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PATHOLOGICAL ANATOMY OF EXPERIMENTAL THROMBOPENIC PURPURA IN THE DOG *

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The injection of a moderate dose of antiplatelet serum into a dog is followed by an acute attack of purpura lasting approximately 48 hours and ending in a spontaneous recovery. Larger doses are often fatal. The clinical manifestations of the experimental disease resemble closely those of thrombopenic purpura in man.[27, 28] This report is concerned with the gross and microscopic anatomical changes in the tissues and organs of dogs that died or were killed during or after one or more attacks of purpura induced in this manner.

From a survey of the literature one gains the impression that the lesions in thrombopenic purpura are neither specific nor constant.[12, 22, 24] Except for widespread hemorrhage, no significant pathological changes have been found common to all autopsied cases of thrombopenic purpura. Perhaps more extensive studies of the disease in man will show eventually a pathological picture, the evolutionary stages of which may resemble those found in our animals.

METHODS AND MATERIALS

Twenty-five mongrel dogs from 8–20 kg. in weight and 1–3 years of age were employed. The antiplatelet serum was prepared

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in the rabbit by 6 intravenous injections of washed platelets isolated from 110–120 cc. of blood from normal dogs.[27] The antiserum thus prepared is of approximately 1:32 to 1:64 intradermal purpurigenic titer. It was administered in varying doses intraperitoneally or intravenously.

Experiment 1: (Fatal Group, 5 Animals): Three were given a large dose of antiplatelet serum intraperitoneally (1–2 cc. per kg. body weight), and all died spontaneously 1, 1 and 3 days later, respectively. The other 2 animals died 1 and 2 days, respectively, after a moderate dose (0.1 cc. per kg. body weight).

Experiment 2: (Serial Group, 12 Animals): All were given a moderate dose of antiplatelet serum intraperitoneally (0.1 cc. per kg. body weight) and killed 1, 2, 3, 4, 5, 7, 10, 12, 13, 15, 17 and 34 days later respectively.

Experiment 3: (Intravenous Dose Group, 4 Animals): All were given a moderate dose of antiplatelet serum intravenously (0.1 cc. per kg. body weight) and killed 2, 17, 22 and 28 hours, respectively, afterwards.

Experiment 4: (Multiple Dose Group, 2 Animals): One received 2 large doses (1–2 cc. per kg. body weight) of antiplatelet serum intraperitoneally 5 days apart, and was killed 23 days after the last injection. The other received 7 moderate doses (0.1 cc. per kg. body weight) at 3 day intervals and was killed 33 days after the last injection.

Experiment 5: (Control Group, 2 Animals): These were given injections of normal rabbit serum in amounts equivalent to a moderate dose of antiplatelet serum. The injections were given intravenously and intraperitoneally, respectively, the animals being killed 5 and 24 hours later.

In the body of the text these experiments are designated as the fatal, serial, intravenous dose, multiple dose, and control groups. The expression "acute phase of purpura" indicates that period of the experimental disease lasting approximately 48 hours, during which the bleeding time is prolonged and hemorrhages develop at sites of trauma.

The dogs were killed over a period of 15 to 30 minutes by bleeding from the carotid artery after infiltrating the tissues locally with procaine. The autopsy was performed immediately afterwards and pieces of the various tissues were placed in fixing fluid as soon

as possible. The autopsy was complete in all respects with the exception of the spinal cord. The organs and structures of the abdominal, thoracic, cranial, orbital, oral and nasopharyngeal cavities were examined, together with the structures and organs of the face and neck, superficial lymph nodes, cutaneous, muscular, peripheral, nervous and osseous tissues, and bone marrow from ribs, sternum, femur, humerus and vertebra. When the animal died of the disease it was not always possible to carry out the autopsy immediately after death, but in the study of the tissue sections ample allowance was made for the possible complicating factor of postmortem autolysis. Likewise, due consideration was given the observation of others [8] that a few small hemorrhages may occur spontaneously during the agonal period when methods of killing somewhat analogous to ours are employed. That this is a small factor contributing to the production or aggravation of hemorrhagic manifestations is attested to in our control animals which showed only a few petechiae in the splenic mesentery, peritoneum of liver, renal capsule and mucosa of ileocecal junction. In the animals injected with the antiplatelet serum a few apparently fresh petechial hemorrhages, which occurred long past the acute phase of purpura, might thus have been agonal in character.

Pieces of tissue were fixed in a solution of formaldehyde (U. S. P., 1:10), and in Zenker's fluid; some were frozen and sectioned and stained for fat; the rest were embedded in paraffin and cut and stained by the following methods: hematoxylin and eosin, hematoxylin azure eosin, methylthionine chloride (U. S. P., methylene blue), van Gieson's and Mallory's connective tissue and Verhoeff's elastic tissue stains, and Mallory's potassium ferrocyanide stain.

Inasmuch as these methods of fixation and staining do not enable one to differentiate in tissue sections between platelets and platelet-like matter, no conclusions were drawn in regard to the changes and distribution of these elements throughout the body. Without resort to special methods,[29] platelets may be confused with precipitated plasma protein, pigment granules, disintegration products of blood cells, fibrils of connective tissue and pseudopodia of reticulum cells, fibrin strands and products of cytolysis.

Experimental Observations

Skin and Mucous Membranes: Wine colored petechiae and hemorrhages of various sizes (2–40 mm. in diameter) occurred in the skin, especially about the mouth, ears, breast, groin, axilla and scalp, and at points of trauma, as cuts and needle punctures. Absorption of the hemorrhages, as indicated by fading, began shortly after their development and although a few were found as late as the 17th day, by the 4th day their number was materially reduced. There was a marked delay in this absorption in the fatal cases. In the early stages of purpura blood-stained discharges issued from the nose, mouth, rectum and vagina and the mucous membranes of these structures showed congestion, edema, petechiae and hemorrhages, the latter being numerous about the gum margins, base of tongue and tonsils. At this period there was usually a moderate degree of subcutaneous edema and in 1 animal that died from a large dose this was so marked that one foreleg was more than twice the size of its contralateral. Collections of edema fluid were distributed around hair follicles and small vessels of the papillary layer and caused separation of the connective tissue fibers of the skin. Hemorrhages were generally superficial in the derma but some extended deeply subcutaneously and along the ducts of the mammary gland. The erythrocytes were fairly uniformly distributed in parallel rows between the tissue fibers. Hemolysis and erythrophagocytosis occurred but fibrin was almost always absent. Occasionally, a slight inflammatory reaction was observed with small foci of necrosis in the overlying epidermis. In the tonsils of the animals of the intraperitoneal group the hemorrhages were small and confined to the stroma between the follicles, but in those of the intravenous doses group they were extensive both within and between the follicles.

Skeletal Muscle: Muscular hemorrhages developed in all animals within the first 17 days of the experimental period. The extravasated blood was distributed in longitudinal streaks parallel to the muscle fibers. During the acute phase of purpura the individual muscles were often notably increased in volume as a result of a combination of edema and hemorrhage. The areas most frequently involved were the tongue, musculature of the anterior thoracic and abdominal wall, diaphragm, and intercostal and axil-

lary muscles. Subperiosteal hemorrhages were frequently observed, especially over the calvarium and at the costochondral junction. In muscle the erythrocytes were either sparsely distributed or collected in large areas showing associated degeneration and necrosis of the muscle fibers and infiltration of inflammatory cells. In the tongue, extravasated blood appeared to spread dorsally, compressing the muscle fibers and blood vessels; the lymph vessels, however, were distended and filled with red staining material. Even in the absence of hemorrhage small groups of swollen hyalinized muscle fibers and minimal inflammatory changes were found in the extraocular, pharyngeal, laryngeal and tracheal muscles as late as 23 days after an injection.

Heart and Aorta: Cardiac lesions were observed in all animals. In the early cases the myocardium was soft, flabby, degenerated, edematous and mottled, dark red, gray and yellow. Fluid blood and different types of postmortem clots were found in the chambers of the heart, which were, in a few instances, distended to an unusual degree. During the first 12 days hemorrhages were common, particularly in the fatal cases. The pericardial fluid was sometimes blood-tinged and petechiae were numerous in the parietal pericardium and in the connective tissue around the aorta. In the epicardium petechiae and red linear streaks were frequently distributed along the course of the coronary vessels, the smaller branches of which were often congested. Subendocardial hemorrhages tended to occur at the base and free margins of the valve leaflets and superficially on the crests of the muscle bundles (Fig. 4), diminishing in the ventricles from base to apex. In 1 animal the entire auricular wall was hemorrhagic. In the course of time (7th to 34th days), these hemorrhages lost their bright color, turning brown or green, and underwent absorption. In the more recent lesions the erythrocytes were distributed singly and in groups loosely or tightly packed in and beneath the endocardium and epicardium, in the connective tissue septums, between individual muscle fibers, around blood vessels and in the lymphatic vessels. At various stages of absorption there were hemolysis, pigmentation, infiltration with leukocytes, erythrophagocytosis and occasionally a deposit of fibrin. Of particular interest were the endocardial bleb-like hemorrhages (Fig. 5) situated on the crests of the muscle bundles forming the chambers. The overlying endo-

thelial cells were tightly stretched but their continuity was nowhere broken. Beneath the blebs the connective tissue was edematous with focal hemorrhagic and inflammatory changes. All the animals studied showed some degree of swelling and vacuolization of the superficial muscle fibers near the endocardial surface. In several, during the acute phase of purpura, degenerative and necrotic changes involving groups of 3–20 muscle fibers were observed throughout the myocardium. In a fatal case there was a diffuse interstitial inflammatory reaction associated with extensive degenerative and necrotic changes in the muscle (Fig. 6). Another animal killed 23 days after the last of several doses showed extensive areas of chronic granulation tissue and fibrosis together with degenerative and necrotic changes in the muscle fibers. No changes were observed in the aorta.

Lungs and Pleura: In only a few of the animals the pleural cavity contained a slight excess of fluid and this was rarely blood-stained. During the acute stage the small intrathoracic vessels were congested and hemorrhages of varying extent were observed in the pulmonary tissue, coats of the great vessels, esophagus, trachea, bronchial lymph nodes and visceral and parietal pleura. In the fatal group and those killed early the pulmonary tissue was extensively hemorrhagic with varying grades of atelectasis, emphysema, edema and bronchopneumonia. In 1 animal there was a diffuse interstitial pneumonitis and in another a fatal massive submucous tracheal hemorrhage half obstructing the lumen.

Scattered over the visceral pleura of both lungs, especially along the margins, were numerous hemorrhagic areas, button-like or sometimes bleb-like, slightly elevated, and from 1–10 mm. in diameter (Fig. 10). Some presented a uniformly bright red stippled appearance with radiating streaks while in others the center was pale blue or purple with a surrounding red zone. These button-like lesions were most numerous during the first 5 days but were observed in various stages of regression and decolorization as late as 23 days after injection. The microscopic appearance of this lesion was that of any emphysematous, hemorrhagic pneumonic area embedded in atelectatic lung tissue. There were many eosinophils in the exudate. Involution was accompanied by erythrophagocytosis, hemolysis and pigmentation.

The most striking pulmonary lesion consisted of collar-like

extravasations of blood encircling bronchi and pulmonary vessels. It occurred regularly during the first 2 days. Not only was the lesion absent after the 2nd day, but there were few signs suggesting its previous presence. In some animals every branch and twig of the bronchial and blood vascular trees were involved but generally the lesion was confined to the medium sized subdivisions. The vessels and tubes thus surrounded showed slight compression but practically no irregularity or distortion of the lumens. When the thus affected bronchi were opened, the mucosa appeared dark bluish to black in the smaller ramifications. Around the blood vessels these hemorrhagic extravasations occupied a sharply delimited field in the adventitia and surrounding connective tissue, leaving the medial and intimal coats unaffected (Figs. 12 and 14). At the outer limits of the area was a thin fibrous and elastic membrane sharply delimiting the surrounding, slightly compressed pulmonary tissue. In the bronchi the ring was less often complete and erythrocytes were scattered throughout the remainder of the bronchial wall and in the lumen. The lesion almost never accompanied the smaller thin walled branches issuing from affected parent vessels; under these circumstances the collar-like extravasations stopped abruptly at the junction point. In the fresh state these lesions were composed of well preserved packed red cells, connective tissue fragments, and later macrophages showing active erythrophagocytosis. Widely spaced strands of connective tissue were arranged radially around the bronchus and circularly around the blood vessels. The bronchial mucous glands and occasionally the surface epithelium were compressed and necrotic.

On the 4th and 5th days the pulmonary vessels showed extensive clot formation and marked swelling of the endothelial cells. The pulmonary tissue, however, showed no evidence of recent infarction at this stage. Only 1 dog, at the 34th day, showed an organized infarct associated with canalized thrombosed blood vessels. Megakaryocytes were found in 3 animals of the serial group sacrificed on the 10th, 12th and 13th days and were exceedingly numerous in the small veins and capillaries. Their nuclei were hyperchromatic and distorted and processes of the cytoplasm radiated to the vessel wall, giving the cell a vacuolated appearance.

Gastro-Intestinal Tract: Hemorrhagic manifestations were abundant in the visceral and parietal peritoneum, omentum, mesen-

tery and alimentary tract in the acute phase of purpura and were observed, in diminishing frequency, up to 33 days. Blood tended to extravasate at the visceral attachments of the omentum and mesentery and along the veins (Fig. 2) which often exhibited ampulla-like dilatations. Intraperitoneal hemorrhagic effusions (from 10–250 cc.) were occasionally observed. The course traversed by the injection needle through the abdominal wall was surrounded by blood and the inner aspect of the abdominal wall at this point was marked by a localized area of fibrinohemorrhagic peritonitis which later organized with the formation of a large granulomatous mass. The majority of the hemorrhages in the esophagus, stomach and intestine were in the mucosa and submucosa but sometimes all coats were involved. Hemorrhages were most numerous in the mucous membrane of the pyloric ring, ileocecal valve, cecum and appendix (Fig. 1), and were absent from the Peyer's patches except in a single animal. Clusters of petechiae over an area 0.5–3 cm. were situated on the crest of the mucosal folds and sometimes in the grooves between the rugae (Fig. 3) or in a linear arrangement in the rectum. When there was hemorrhage in the submucosa or stroma of the intestinal glands the lining epithelial cells often showed degeneration, necrosis, desquamation and mitosis. Deeper hemorrhages were sometimes surrounded by necrotic muscle fibers and inflammatory cells.

Massive areas of hemorrhage involving large portions of the wall of the stomach or intestines were observed in 7 animals, 5 during the acute phase of purpura and 2 on the 5th and 13th days respectively (Fig. 2). The intestinal areas were situated 50–76 cm. from the pyloric orifice, measured 4–6 cm. in length, and were firm, mottled, dark red and swollen and occasionally obscured by local adhesions. All coats of the intestine were permeated with blood and the overlying peritoneum was elevated, stretched and torn in places. The mucous membrane was swollen, soft and deep red. Although the lumen of the intestine at the level of the lesion was usually markedly constricted, there was little or no change in its caliber above this point. Distally the lumen was distended and the contents blood-stained. In the stomach the lesion was massive and most of the hemorrhagic extravasation occurred into the submucosa and subserosa of the anterior wall. In 1 animal there was an associated intussusception involving the anterior wall of

the stomach, the ensheathing segment (intussusceptiens) being formed by the hemorrhagic portion of the lesion into which the immediately proximal gastric wall was telescoped. Microscopically there was extensive necrosis of muscle fibers, nerve cells and overlying mucous membrane, marked inflammatory changes, and recent and canalized thrombi. The red blood cells were gradually broken down and absorbed. A later stage of this lesion was observed in a 13 day serial group dog; at this time most of the blood had been absorbed and large areas of muscle had been replaced by fibrous tissue.

Thymus: Petechiae, edema and venous congestion were observed in and around this organ chiefly during the first 7 days. Hemorrhage tended to occur in the interlobular and perithymic connective tissue, rarely within the lobule itself. Following these acute manifestations there was a marked proportionate numerical increase of reticular cells over thymocytes, and extensive pigmentation of phagocytes.

Adrenals: Only minor changes were noted in these organs. During the acute stage of purpura there was congestion of the vessels at the corticomedullary junction, hemorrhages in the surrounding areolar tissue and occasionally a few small hemorrhages in the medulla.

Thyroid: During the acute stage of purpura there were edema and congestion; small acinar collections of blood were observed in 2 dogs on the 5th and 12th days respectively.

Salivary Glands: Gross and microscopic study of these organs yielded negative results.

Spleen: Grossly not many changes were observed except for hemorrhages in the mesenteric attachment and, after the 10th day, follicular hyperplasia due chiefly to a marked increase in the large pale mononuclear cells of the germinal centers. In 2 animals killed on the 12th day and 13th day practically every follicle throughout the spleen contained a sharply circumscribed oval or crescentic red area composed of fairly well preserved erythrocytes and a few mononuclear phagocytes without associated changes in the surrounding pulp and sinusoids (Figs. 15 and 16). In the acute stage wine colored fluid blood issued from the cut surface. On the 5th day the large vessels contained cellular clots. From 4 to 15 days after an injection intravascular plugs were regularly

observed in the majority of the large and small vessels, frequently occluding their lumens to a considerable extent (Fig. 17). They were closely approximated to the vessel wall and were composed chiefly of large numbers of leukocytes with a variable but smaller proportion of erythrocytes and platelets, with fibrin in two instances and megakaryocytes in one. It was never possible with certainty to regard these "clots" as ante mortem, agonal or post-mortem. They were closely approximated to the vessel wall and platelets were arranged festoon-like as occurs in a thrombus. On the other hand, there was never any evidence of coincident infarction or congestion of the splenic tissue, no changes in the vessel wall along the line of approximation and no organization or canalization of the masses.

Megakaryocytes appeared from the 3rd to the 15th day in increasing numbers, averaging sometimes from 12 to 30 per low power field. They were of the young and old type and were located most frequently near the trabeculae, in groups of 2 or 3, in association with erythropoietic foci, or in the lumens of vessels. In 1 dog, dying 1 day after a single large dose, there were several megakaryocytes encircled by large mononuclear cells, as in the bone marrow.

Liver: The hepatic changes differed considerably, depending upon the route of administration of the serum. In the intravenous group the liver was large, edematous and mottled, deep red and gray. Microscopically about the portal radicles there were hemorrhage, intense inflammatory changes, extreme distention of lymphatic vessels (Fig. 8) and compression and necrosis of bile ducts and adjacent parenchyma. The inflammatory cells consisted chiefly of polymorphonuclear leukocytes and eosinophils. The central and sublobular veins were constricted and throughout the lobule there was extensive hyaline and fibrinous thrombosis of the sinusoids with sporadic necrosis of the parenchyma and hemorrhage. Clusters of erythrocytes and large spherical hyaline droplets were observed in rounded spaces interpreted as bile canaliculi between hepatic cells. There were petechiae and hemorrhages in the serosa of the bile ducts and gall bladder, and the wall of the latter was thickened by an exudative inflammatory reaction (Fig. 13).

In the animals that received the serum intraperitoneally the

acute changes resembled those just described but were milder in character and of gradual development and regression. Other changes included blood in the bile ducts, portal vein thrombi and, from the 7th to the 12th day, small foci of erythropoiesis. As a rule central veins were collapsed and inconspicuous. Small hyaline thrombi were occasionally observed in the sinusoids and a few small hemorrhages about portal radicles. Parenchymal necrosis occurred throughout the first 15 days, being extensive in the first 3 days and located chiefly in the inner two-thirds of the lobule, frequently in association with sinusoidal thrombi and hemorrhages. Regressive changes were accompanied by hepatic cell regeneration which was especially active on the 4th day. The lesions usually underwent complete resolution; in only 1 animal, killed at the 34th day, was there a moderate portal fibrosis.

Pancreas: No lesions were observed except slight congestion and a few interstitial petechiae.

Genito-Urinary Tract: During the first 4 days the kidneys showed a variable degree of nephrosis and a few petechiae in the capsule and perirenal fat. Subserous, submucous and intramural petechiae and hemorrhages were frequently found in the bladder, especially in the region of the trigone. In the male dogs hemorrhages occurred in the tunica vaginalis, epididymis, vas deferens and testicle during the phase of purpura. The testicular hemorrhages were large and wedge-shaped, extending from the center of the lobule to the periphery. In the female sex organs only a few petechiae were noted in the peritoneum of the ovary and in the uterine mucosa. Vaginal bleeding has been referred to above.

Brain: Intracranial hemorrhage of some degree, usually minimal, was observed during all stages of the experiments, being perhaps in some instances agonal in origin. Hemorrhage was most marked in the fatal cases, yet even in these no extensive extravasations occurred into the brain substance or into the ventricular cavities. Petechiae and larger purpuric patches occurred over the inner surface of the skull, in and beneath the dura, pia and arachnoid, especially along the sulci and Pacchionian bodies, subependymally, in the chorioid plexus and occasionally also in the parenchyma. Superficial parenchymal hemorrhages resulted from extension of the subpial extravasations, whereas the deeper ones were peri-

vascular in location and numbered as many as 3 per low power field. Immediately around the latter there was a narrow zone of tissue degeneration. In the animals of the intravenous injection group the cerebral tissue was edematous, congested, and of soft consistence.

Pituitary: No visible changes were observed aside from vascular congestion during the acute stage of the purpura.

Lymph Nodes: In the acute stage the nodes were large, soft and succulent, and contained red areas outside, in and beneath the capsule, and on the cut surface. Externally these red areas corresponded either to hemorrhages or to distended afferent lymph vessels packed with erythrocytes (Figs. 7 and 9). These red areas were noted in varying intensity in different nodes up until the 17th day. However, many nodes began to show a brownish cast after the 4th day. Within the node were tightly packed erythrocytes in distended lymph sinuses, chiefly in the medulla. The impression was gained that the red cells were brought by afferent lymph vessels, passed quickly through the cortical sinuses, and stagnated in the medulla. The process was always orderly even when large numbers of red cells were present. With marked distention of the sinusoids the intervening medullary cords became atrophic. The erythrocytes were cleared away by phagocytosis and the deposition of pigment, followed by an increase in lymphocytes in the still distended sinuses. Pigmentation was greatest between the 10th and 17th days. By the 34th day the histiocytes were comparatively inactive, shrunken and had long thin processes. The compression atrophy of the lymphoid tissue during the acute phase was followed (from the 10th to the 34th day) by hyperplasia of the medullary cord and follicles, the latter being composed of large pale cells with a reticular nucleus. In all the sections examined only 3 megakaryocytes were found (on the 12th and 34th days).

Bone Marrow: Changes in the megakaryocytes were the principal feature observed in this tissue, thus reflecting the close association between platelets and these giant cells. Hyperplasia and varying predominance in quantity of erythroid and myeloid elements did not differ significantly from the controls. Microscopic hemorrhages developed only in the fatal cases and, in association with thrombi, in a 2 hour intravenous group dog. The

megakaryocytes showed combined regressive and regenerative features both in the series as a whole and in the same animal. Pyknosis, karyorrhexis and karyolysis, marked irregularity in cell outline and increased granularity of the cytoplasm involving 50 per cent or more of these giant cells predominated from 2 hours to 15 days; later than this necrosis was rare. What appeared to be invasion by polymorphonuclear leukocytes was present at all stages, being most marked at the 13th day when 30–40 per cent of the megakaryocytes were so affected; as many as 9 polymorphonuclears were counted in a single megakaryocyte. Young megakaryocytes were observed in greatest number 2 hours after an intravenous injection. In the serial group they increased numerically from the 2nd to the 13th day (Fig. 20), after which they began to decline. A few forms interpreted as mitotic figures were observed in all stages of the experiments.

Another mechanism for the formation of bone marrow giant cells distinct from the generally proposed one was suggested by a curious grouping of mononuclear cells about single necrotic megakaryocytes in a circular fashion resembling a rosette figure. These figures were observed in 5 dogs at the 2nd and 3rd day in the serial group and at 17, 22 and 28 hours in the intravenous group. The marrow of these animals showed a diminution in the myeloid and erythroid elements and a diffuse increase in a mononuclear type of cell with a large, pale vesicular nucleus and relatively little cytoplasm. Many of these mononuclear cells were also arranged focally around megakaryocytes in a rosette form outside the cell border, completely isolating them from other cells (Figs. 18 and 21). The nuclei of the encircled megakaryocytes were pyknotic, irregularly shaped and indented, and the cytoplasm vacuolated. Many phases of this process were observed from mere accumulation of a few cells around apparently viable megakaryocytes to the point where the nucleus of the latter existed only in outline encircled by several cell rows. In some marrow specimens the majority of the megakaryocytes were necrotic and encircled by these mononuclear cells. In addition to these formations there were numerous giant cells with concentrically arranged nuclei (Fig. 19), with hypertrophied and apparently fused nuclei, representing perhaps transitional stages in the formation of mature megakaryocytes.

Comment

The lesions of experimental purpura differ somewhat according to the method employed for inducing it (intravenous or intraperitoneal injection) and with the stage of the disease. When the antiplatelet serum is administered intravenously the hepatic changes indicate an anaphylactic response resembling that produced by other types of cytotoxic antiserums.[25, 32] Following intraperitoneal injection, however, the serum is absorbed more slowly, the number of platelets in the circulation diminishes more gradually, and the resulting tissue changes are probably more comparable with those in naturally occurring thrombopenias in man. The clinical and pathological manifestations of the experimental disease show a uniform evolution, the stages of which may be conveniently divided as follows: (1) acute stage (1–5th day), exhibiting thrombopenia, prolonged bleeding time and, in the tissues, hemorrhage, edema and pigment deposition; (2) intermediate stage (5–10th day), showing a rising platelet count, short bleeding time, and multiple vascular thrombi in various organs, principally in the spleen; (3) reactive stage (after the 10th day), characterized by a high blood platelet count, follicular hemorrhages in the spleen and hyperplastic changes in the bone marrow, spleen, lymph nodes, thymus and Peyer's patches of the ileum.

There have been few studies of the lesions of thrombopenic purpura in man in which proper consideration was given to such features as the date of onset and duration of the disease, the number of attacks, the degree of thrombopenia, the distribution and intensity of the hemorrhages, and the mechanism of the period of recovery, and so on. There are sporadic indications that the evolutionary changes of experimental purpura in the dog are duplicated, to a certain extent, in man. Thus in a patient with thrombopenia complicating lupus erythematosus, Baehr and coworkers [3] noted occlusion of nearly all precapillary arterioles with platelet thrombi. Klemperer [23] observed thrombi in the arteries of all organs of a girl with thrombopenic purpura who died after a splenectomy and, in another patient, reticulum cell hyperplasia of the spleen with widening of Billroth's cords. Arrigoni and Calabresi [1] observed cellular adherent thrombi in the veins and hyperplasia of the reticuloendothelial cells in the spleens of their

patients. Perivascular extravasations of blood from the vessels of the brain forming ring-like hemorrhages are characteristic of "cerebral purpura" complicating arsphenamine therapy.[15] The occurrence of follicular hemorrhages in the spleen has been observed by us and others.[5, 7] The frequency of hepatic edema in thrombopenic purpura has been recently emphasized; in 55 per cent of such cases studied by Keschner and Klemperer [22] there was collection of fluid between the liver cell cords and the walls of sinusoids. Acute abdominal symptoms may complicate purpura in man,[20] sometimes eventuating in a laparotomy [4]; in these cases there are often petechiae and ecchymoses in the mesentery and the appendix is enlarged, edematous and hemorrhagic, a counterpart of the manifestations of early experimental purpura in the dog. Intestinal intussusception with obstruction is not a rare complication in man.[16]

From this evidence it seems likely that the lesions of thrombopenic purpura, as a group at least, are more specific than is generally believed. It is admittedly difficult, however, to establish these correlations solely from human pathological material. Most patients with primary thrombopenic purpura recover spontaneously from the acute attack and in fatal cases little or no allowance is generally made for the duration of the disease in evaluating tissue changes. Moreover, if, as frequently happens, the thrombopenia is secondary, its lesions are masked to a variable degree by the primary condition, be it leukemia, aplastic anemia, carcinoma, or tuberculosis or other infections. Recurrent attacks at short intervals result in superimposed lesions and a more complex histopathological picture. By making serial observations in experimental animals over the period of evolution of a single attack, some of these pitfalls may be avoided.

Certain deductions may be made from a study of the distribution and extent of the hemorrhagic manifestations and their relationship to preexisting and concurrent lesions, trauma, mobility and vascularity of parts. Trauma plays a major rôle in conditioning the skin for the immediate or delayed development of local hemorrhages.[13, 27] In animals injected with antiplatelet serum hemorrhages develop at points in the skin traumatized within 4 days preceding the injection, as well as during the acute stage of purpura.[27] Essentially similar conditions obtain in the internal

viscera. For example, no hemorrhages were found in and about long-standing chronic granulomatous lesions of a parasitic nature in parenchymatous organs, on serous surfaces or in the musculature, especially of the tongue; at points of attachment of intestinal worms hemorrhages were sometimes present, sometimes absent, depending in all probability on whether the trauma was recent or remote. A tendency exists for hemorrhages to occur in organs that normally during life are undergoing movement almost constantly or being subjected to external or internal changes in pressure, even while the subject is at rest. Thus, hemorrhages were common in the lung, thymus, heart, intestine, mesentery, omentum and urinary bladder; they were minimal in relatively immobile or protected organs such as the bone marrow, pancreas, adrenal, liver, kidney and brain. Local trauma, stress and spontaneous lesions, both recent and remote, seem, therefore, to play an important part in the localization of hemorrhages in purpura. Examples of normal internal stresses that may constitute mild forms of trauma are the alternating intrapleural and intracardiac pressure, action of the heart, intestinal peristalsis, and contraction and distention of the urinary bladder. Under normal conditions these stresses are adequately compensated and no ill effects result; in thrombopenic purpura or in scurvy [2, 33] compensation fails and hemorrhage may result. If, as is frequently maintained, the effects of the antiplatelet serum were due principally to its injurious action on blood vessels, then one would expect to find the hemorrhages distributed more in proportion to the vascularity of the organs and to occur frequently, for example, in the adrenals, kidney or epididymis. Such was not the case, however, for in these organs hemorrhages were rarely seen.

The mechanism of hemorrhage in purpura, the rôle played by the vessels, and the point in the vascular tree through which blood escapes, have long been the subject of speculation. According to Unna,[30] the cutaneous extravasations of blood originate principally from ruptured venules in the subpapillary layer. We have been unable to confirm this observation. The relationship between an area of extravasated blood and any given vessel may be purely coincidental. Curious appearances may be presented in single histological sections by such structures as the neurovascular glomus body. Breaks in the walls of small and large blood vessels

were repeatedly observed in the material from both the purpuric and the control animals and were in many instances obvious artefacts. Any hypothesis designed to explain the escape of blood into the tissues must take into account the fact that under physiological conditions erythrocytes may traverse the apparently uninjured capillary wall.[11] It follows that an exaggeration of this mechanism in purpura may lead to increased diapedesis of erythrocytes, independent of injury to the endothelium or rupture of the wall of the vessel.

The most unusual type of hemorrhagic lesion encountered in this series of animals occurred in the lung during the first days of thrombopenia in the form of peribronchial or perivascular circular accumulations of blood. We interpret these lesions as representing blood within the adventitial and perivascular lymphatic vessels. In other words, the erythrocytes thus situated occupy a position analogous to that of exogenous pigment granules and associated phagocytic cells in certain forms of pneumoconiosis (Fig. 11). The blood was absent from these areas after 48 hours and there was no evidence of fading such as ordinarily occurs in most hemorrhages. The distended, filled lymphatic vessels may be seen clearly when this lesion is observed in newborn puppies with purpura induced by antiplatelet serum. In the adult dog the outlines of the lymphatic vessels are less readily demonstrable. Several factors may be contributory to the production of this lesion: increased absorption by the lymphatics of red cells from hemorrhagic areas in the periphery of the lung, exaggerated diapedesis of erythrocytes, or lymph stasis due to overburdened regional lymph nodes with retrograde flow of lymph. That the lymphatics play a dominant rôle in the pathogenesis of this lesion is attested by the fact that afferent lymphatic vessels to lymph nodes were likewise often greatly distended with blood (Figs. 7 and 9). Against the possibility that the blood in the collar-like lesion issued from the vessel so surrounded or from the adjacent pulmonary tissue is the sharply demarcated character of the lesion (Fig. 12). Furthermore, the usual failure of hemorrhages in general to show absorption in the center, in the animals that died from an acute attack, may be another sign of the retardation or failure of lymphatic circulation. Congestion and stasis of lymphatic vessels may also account for the edema which is a

constant finding in the acute phase of purpura. Marked distention of lymph vessels was observed not only about lymph nodes in the perivascular pulmonary tissue but also in the portal radicles of the liver and in loose connective tissue, especially of the mesentery and omentum.

A connection may exist between these collar-like pulmonary hemorrhages in the dog with induced purpura, and the occurrence of clinical purpura in cases of lymphangitic carcinomatosis of the lungs. The latter condition has been recently reviewed by Jarcho.[19] Almost all of the thoroughly studied cases of carcinoma with purpura have been characterized by lymphangitic carcinomatosis of the lung.[19] Despite the fact that many such cases also exhibit neoplastic permeation of the bone marrow, the accompanying thrombopenia cannot be linked to destruction or disappearance of megakaryocytes; on the other hand, practically all such cases of purpura show neoplastic permeation of the lymph vessels of the lung. In view of the evidence for the pulmonary origin of platelets,[18] one may venture to speculate regarding the effect of lymphangitic carcinomatosis of the lung on thrombopoiesis. The primary tumor in these cases is usually situated in the stomach.[19] It has been suggested that tumor cells emanating from this organ proceed to the abdominal lymph nodes, thence to the mediastinal nodes, and thus engender a stasis of the lymph in the lungs. Similar routes may have been traversed by products of the local purpuric lesion in the region of the stomachs of our animals injected intraperitoneally with antiplatelet serum, thus perhaps explaining the similarity in the morphology of these two pulmonary lesions.

None of the spleens in our animals presented enlargement, congestion or internal hemorrhages during the acute phase of purpura despite the fact that this organ displays a certain degree of movement. Killing by exsanguination may, of course, have reduced the size of a previously engorged spleen. A palpable spleen is occasionally found clinically in thrombopenic purpura and has been attributed to hyperplasia or to accumulation of blood in the pulp by either sinusoidal congestion or massive extravasation. In our animals follicular hemorrhages occurred on the 12th and 13th day, when the number of platelets in the peripheral blood reaches its highest level. We have observed at autopsy an

identical lesion in a female subject in whom there were widespread hemorrhages associated with a long-standing clinical erythroleukothrombopenia. Carnot, Lafitte and Fiehrer [7] reported a similar case of profuse bleeding checked by removal of the spleen in which follicular hemorrhages were found, the intervening pulp being only moderately congested but otherwise normal. This was the sole lesion found in 1 of 11 spleens from patients with thrombopenic purpura examined by Brown and Elliott.[5] In a group of dogs injected with massive doses of parathyroid hormone follicular hemorrhages of the spleen were a constant finding, along with hemorrhagic manifestations in other parts of the body.[6] The etiology and mechanism of development of these splenic hemorrhages is obscure. It is difficult to understand their occurrence in the dog at a time when the animal has recovered from an acute attack of purpura, the mean bleeding time being shorter than normal and the number of platelets in the blood increased. Since the spleen plays an important rôle in the distribution, utilization and, indirectly, in the formation of platelets, the occurrence of these follicular hemorrhages might be linked, in the dog, to the excessive number of platelets, but in man such association seems unlikely. The attack of bleeding in the patient studied by Carnot and coworkers had lasted 6 weeks before splenectomy, in the one reported by Brown and Elliot, 3 weeks, and our patient died after several months hospitalization during which the platelets at repeated determinations did not at any given time go beyond 50,000 per cmm.

Qualitative changes in the megakaryocytes have been stressed as being equally as important in influencing the production of platelets as quantitative deficiencies. Thus, megakaryocytes of the young or lymphoid type are considered to possess limited capacity for thrombopoiesis.[26, 14, 17] It is frequently impossible to establish in human thrombopenic purpura any significant direct correlation between the number of megakaryocytes of the bone marrow and the circulating blood platelets. Such a correlation was suggested by our experimental results in the adult dog, the tissue megakaryocytes undergoing marked degenerative changes during the acute stage and seeming to become more numerous after the animal recovered from the attack. At this period young megakaryocytes and some forms suggesting the mode of formation

of these bone marrow giant cells were common. That megakaryocytes may be formed by fusion of adjacent cells is maintained by Di Guglielmo [9] and Fabris.[10] In animals rendered thrombopenic with pyrodine the latter found megakaryocytes resembling closely those we describe as "rosette" forms. Great caution, however, is necessary in drawing conclusions regarding the morphological appearance of megakaryocytes in single histological sections. From a study of serial sections and reconstruction models of individual megakaryocytes Kaufman [21] pointed out that their small end segments may be interpreted as mononuclear cells or young megakaryocytes. When their pseudopodia encircle other cells, like leukocytes, the impression of phagocytosis or invasion may be gained from simple transverse sections.

In our experience the most striking demonstration of the histogenetic relation between the megakaryocytes and the platelet was in 2 day old pups that received a small dose of antiplatelet serum.* Normally in the liver, spleen and bone marrow of such pups there are large numbers of megakaryocytes as well as foci of erythropoiesis and leukopoiesis. The intraperitoneal injection of antiplatelet serum is followed within 24 hours by the practically complete disappearance of megakaryocytes from these three tissues, leaving the foci of erythropoiesis and leukopoiesis relatively unchanged. Megakaryocytes reappear in the bone marrow 3 days following the injection, are fairly numerous by the 5th day and increase in number subsequently.

Summary

The clinical and pathological manifestations of experimental purpura as produced with antiplatelet serum in the dog undergo a uniform evolution, the stages of which may be conveniently divided as follows: (1) acute stage (1st–5th days), exhibiting thrombopenia, prolonged bleeding time and, in the tissues, hemorrhage, edema and pigment deposition; (2) intermediate stage (5th–10th days), during which there is a rising platelet count, short bleeding time, and multiple vascular thrombi in various organs, principally in the spleen; (3) reactive stage (after the 10th day), characterized by a high blood platelet count and

* Many other lesions of experimental purpura in pups differ materially from those in adult dogs and will form the subject of a separate report.

hyperplastic changes in the bone marrow, spleen, lymph nodes, thymus and Peyer's patches of the ileum. There are indications that these evolutionary changes of the experimental disease are duplicated to a certain extent in spontaneously occurring thrombopenic purpura of man.

The intensity and distribution of the hemorrhages seem to be largely conditioned by the trauma and degree of internal or external stress normally undergone by the part.

Signs of congestion and partial obstruction in the circulation of the lymph are evident during the acute phase of purpura. Lymph vessels in walls of the blood vessels and bronchi of the lung, in the heart muscle, afferent lymphatics and sinuses of lymph nodes draining hemorrhagic areas become greatly distended with blood. Edema and failure of resorption of hemorrhages may be the result of failure of the lymphatic circulation.

Follicular hemorrhage is one of the outstanding lesions in the spleen; it appears only in animals recovering from the attack of purpura and immediately after the time when the number of platelets usually reaches its highest level. Marked degenerative changes take place in the megakaryocytes of the bone marrow during the acute stage; groups of mononuclear cells surround degenerated megakaryocytes, the group eventually enlarging and resembling the mature forms of the giant cells. Intraperitoneal injection of antiplatelet serum in 2 day old pups was followed by the almost complete disappearance of the usually numerous megakaryocytes from the liver, spleen and bone marrow.

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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. The vermiform appendix of a dog killed 3 days after a moderate dose of antiplatelet serum intraperitoneally.

FIG. 2. Large intestine of a dog that died 1 day after the injection of a large dose of antiplatelet serum intraperitoneally with rupture and partial obstruction of the intestine.

FIG. 3. Lower part of the esophagus, stomach and small intestine of a dog killed 3 days after a moderate dose of antiplatelet serum injected intraperitoneally.

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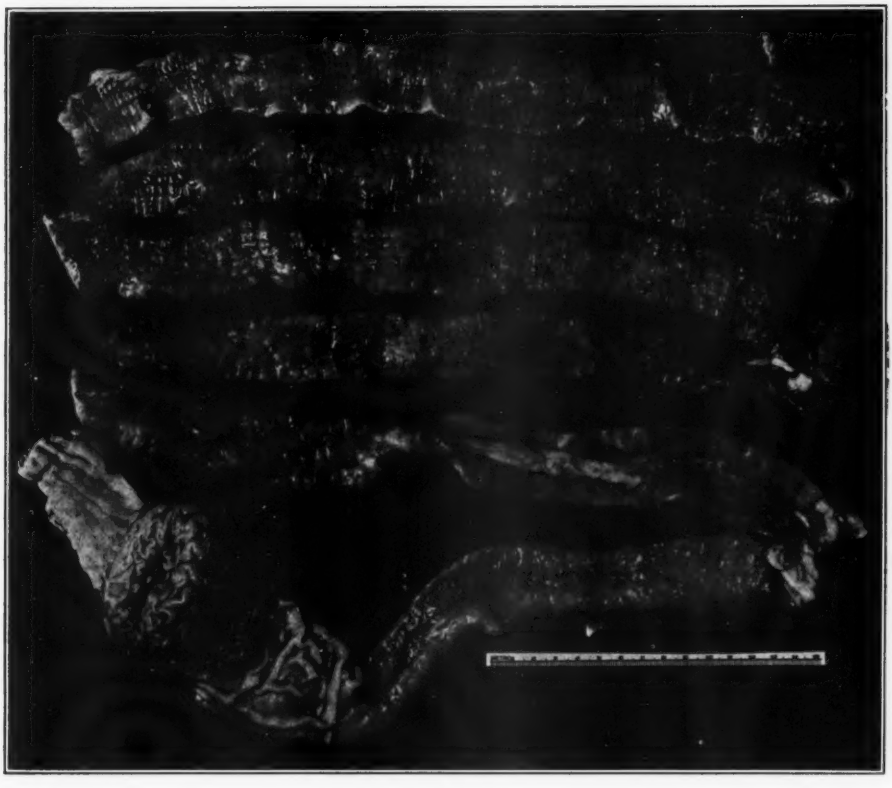

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Tocantins and Stewart Experimental Thrombopenic Purpura

PLATE 2

FIG. 4. A portion of the heart of a dog that died 2 days after the injection of a moderate dose of the serum intraperitoneally.

FIG. 5. Section from the same heart illustrated in Figure 4 showing subendocardial "blood blebs."

FIG. 6. Section from the heart of a dog that died 2 days after the intraperitoneal injection of a moderate dose of antiplatelet serum showing cellular infiltration about necrotic myocardial fibers.

FIG. 7. Section of lymph node of a dog that died 2 days after injection of a moderate dose of antiplatelet serum intraperitoneally. An afferent lymph vessel adjacent to the cortical sinus is greatly distended with red and white cells.

FIG. 8. Section of the liver of a dog killed 22 hours after the injection of a moderate dose of the serum intravenously. Marked dilatation of the periportal lymphatics is seen.

FIG. 9. Section of lymph node of a dog that died 2 days after injection of a moderate dose of antiplatelet serum intraperitoneally. Marked engorgement of an afferent lymph vessel is present. Almost all of the medulla of this lymph node was replaced by blood.

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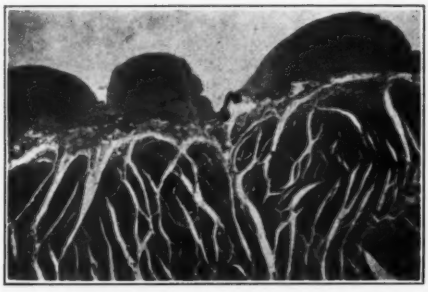

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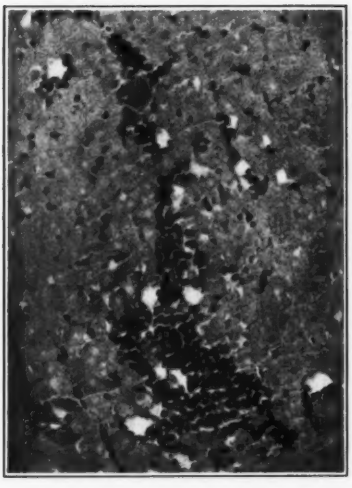

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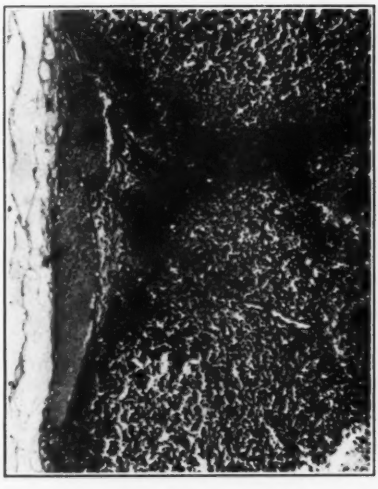

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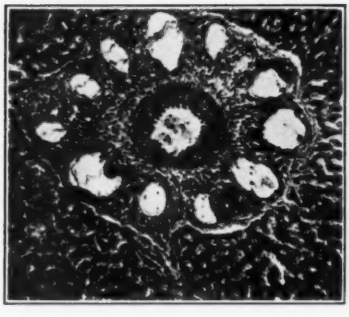

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Tocantins and Stewart

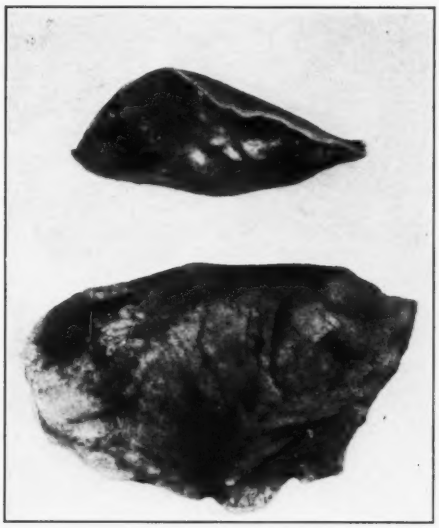
Experimental Thrombopenic Purpura

PLATE 3

FIG. 10. The cut and external surfaces respectively of the lung of a dog that died 2 days after injection of a moderate dose of antiplatelet serum intraperitoneally.

FIG. 11. Low power view of a section of the lung of a dog killed 2 days after a moderate dose of antiplatelet serum intraperitoneally. Massive collar-like accumulations of blood about vessels and bronchi are present.

FIG. 12. Section from the same lung illustrated in Figure 11. High power view of the collar-like collections of blood. The intervening tissue is almost wholly free of blood.



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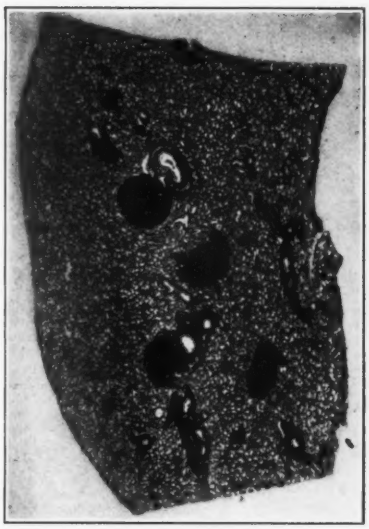

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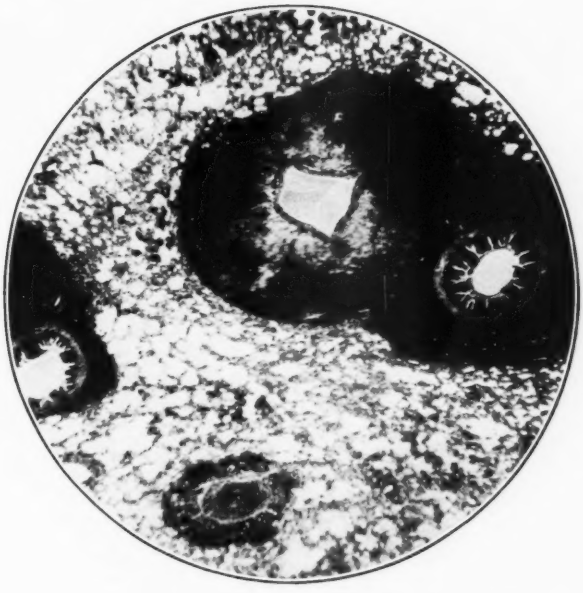

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PLATE 4

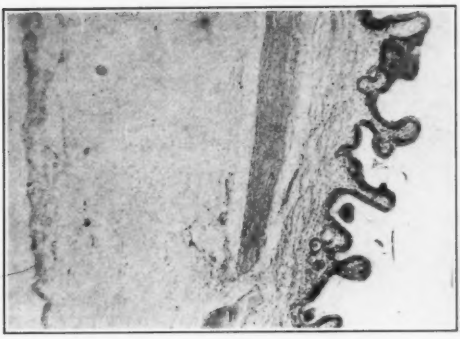
FIG. 13. Section from the gall bladder of a dog killed 2 hours after the injection of a moderate dose of the serum intravenously. Edema of the wall, especially of the serosa, is seen.

FIG. 14. Section from lung of a dog that died 1 day after injection of a large dose of antiplatelet serum intraperitoneally. A massive, collar-like perivascular collection of blood is present which appears to be in the adventitia of the vessel.

FIG. 15. Section from the spleen of a dog killed 13 days after the injection of a moderate dose intraperitoneally showing a perifollicular elliptical hemorrhage around a large germinal center.

FIG. 16. Section from the spleen of a dog killed 12 days after an injection of a moderate dose of antiplatelet serum intraperitoneally showing an intrafollicular hemorrhage.

FIG. 17. Section from the spleen of a dog killed 7 days after the injection of a moderate dose intraperitoneally. An intravascular plug made up of red and white cells and agglutinated platelets is seen.



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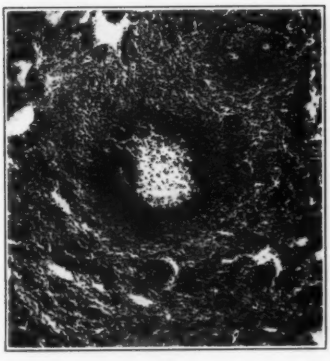

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PLATE 5

FIG. 18. Camera lucida drawing of a section of the bone marrow of the femur of a dog killed 3 days after the injection of a moderate dose intraperitoneally. Megakaryocytes surrounded by mononuclear cells in rosette fashion are seen.

FIG. 19. Camera lucida drawing of a section of the bone marrow of a dog killed 12 days after the injection of a moderate dose of serum intraperitoneally showing megakaryocytes with a circular arrangement of nuclear pieces.

FIG. 20. Section of the bone marrow of the femur of a dog killed 10 days after the injection of a moderate dose intraperitoneally. Numerous megakaryocytes of various types are present.

FIG. 21. Section of bone marrow of a dog killed 4 days after the injection of a moderate dose intraperitoneally showing a megakaryocyte surrounded by mononuclear cells.

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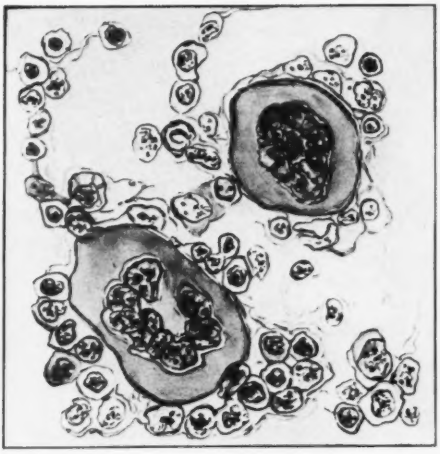

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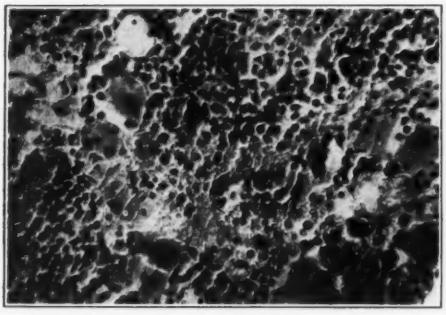

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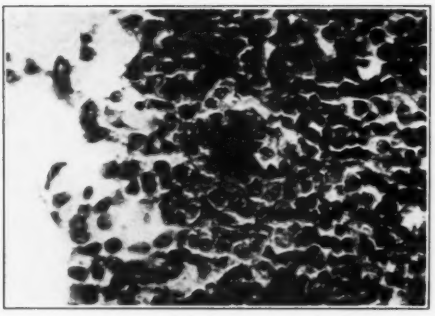

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Tocantins and Stewart

Experimental Thrombopenic Purpura

PATHOLOGICAL CHANGES IN THE HEART, SKELETAL MUSCULATURE AND LIVER IN RABBITS TREATED WITH INSULIN IN SHOCK DOSAGE *

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The main interest in pathological changes produced by the insulin shock treatment since it was introduced by Sakel [1] as a cure for schizophrenia has been centered on the brain. Comparative studies on experimental insulin and anoxic shock revealed a surprisingly close similarity in the symptomatology produced by these two procedures.[2] Rabbits treated with a series of anoxic shocks showed quite definite changes in various parenchymatous organs, in addition to changes in the central nervous system. Therefore, a study of the same organs was undertaken with rabbits exposed to insulin shock. This report concerns mainly the changes found in the heart, skeletal musculature and the liver.

METHOD

Twenty nine rabbits were treated in 4 series with various doses of insulin. Lilly's Iletin was used. Whenever "units" of insulin are referred to, "units per kilo body weight" is meant. The insulin was administered in the morning to rabbits which were not fasting but were, however, not fed on that day until the termination of the experiment. The insulin effect was interrupted between 6 and 12 hours after its administration by feeding cabbage or carrots and pellets. No glucose was given for the termination of the shock except to the rabbits of the first 2 series, which received a subcutaneous injection of 10 cc. of 40 per cent glucose on the 1st day of the experiment just prior to their feeding. In a few other instances glucose was given on various days of the experiment only when the rabbits were obviously unable to recover spontaneously.

The doses of insulin were chosen so that all rabbits of the first 3 series were severely affected, while the doses given to the 4th series remained just below the threshold that had produced deep

* Received for publication September 19, 1938.

coma and convulsions in any instance in the preceding series. Charts 1 to 3 show the doses of insulin administered to single animals of Series I to III, and indicate by broken lines the days on which convulsions were observed. They show also whether the animal died spontaneously or was sacrificed by air embolism or by suffocation in a chamber filled with pure nitrogen.

The 4th series consisted of 8 rabbits treated 28 times with insulin doses of from 0.74 to 1.3 units during a period of 41 days. In addition to this insulin treatment, during the same period these rabbits were exposed 4 times to anoxic shocks, *i.e.* comparatively short periods of gradually increasing deficiency of oxygen in a mixture with nitrogen until the respiration became gasping or ceased entirely. Any accumulation of carbon dioxide in the gas chamber was prevented by continuous flow of the gas mixture. The insulin treatment produced only a very slight apathy in the animals in this series. The anoxic shocks were administered on the 1st or 2nd day, the 8th or 9th, the 17th and the 41st day after the beginning of the insulin treatment. The first 2 anoxic shocks were begun 5 hours after the injection of insulin, the last 2 about 2 hours after the injection. Their duration was from 30 to 60 minutes. Each rabbit was seized by violent convulsions of about 1 minute's duration following the termination of 1 or 2 of the anoxic shocks. At the end of the 1st anoxic shock on the 1st day insulin was injected 1 rabbit was sacrificed, 3 days following the 2nd anoxic shock a 2nd rabbit, and at the end of the 3rd anoxic shock a 3rd rabbit was sacrificed. After the termination of the treatment on the 41st day the remaining 5 rabbits were kept alive for a period of 50 days for full recovery. During this period they showed no pathological symptoms whatsoever. They gained weight from 50 to 300 gm., and were finally sacrificed by suffocation in a nitrogen chamber.

The blood sugar was controlled by the Folin-Wu method on samples of blood withdrawn from the ear vein. In addition, blood was also withdrawn from the hepatic vein immediately after death or several times successively up to 20 minutes after death.

A careful gross examination of each animal was performed after death. Material to be used for frozen sections (fat stains) or the usual staining methods after embedding in paraffin was fixed in formalin (U.S.P.). Thin slices of tissue were fixed in absolute

alcohol for Best's glycogen method which was employed on celloidin or paraffin-celloidin sections.

Results

Individual rabbits displayed a great variability in their response to insulin, as is best reflected by the charts showing by what doses

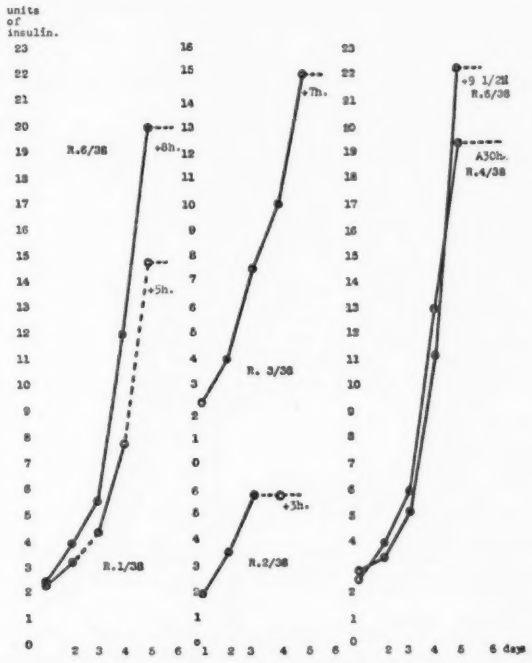

Chart 1. Effect of insulin (units per kilo body weight) on the rabbits of Series I. The broken lines indicate the days on which convulsions were observed.
+ = indicates spontaneous death; A = sacrificed by air embolism; N = sacrificed by suffocation in pure nitrogen. The figure followed by "h" indicates the period of time between the last injection of insulin and death in hours.

and on which days convulsions were elicited. This can be explained only to a slight extent by the fact that the rabbits were not fasted prior to the experiment. Marked muscular weakness and pronounced listlessness and apathy were present, many

times being the sole symptoms even after the administration of large doses of insulin. In some instances the muscular weakness progressed to paralysis of the musculature of the extremities and the neck. Violent convulsions which began suddenly, or after the animal was slightly touched, were noted as a rule from the 3rd to the 6th hour after the administration of an appropriate dose of

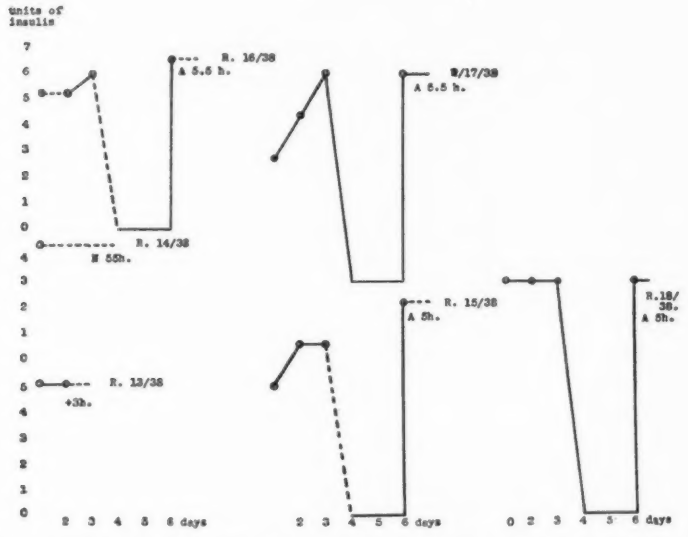

CHART 2. Effect of insulin (units per kilo body weight) on the rabbits of Series II. The broken lines indicate the days on which convulsions were observed.
+ = indicates spontaneous death; A = sacrificed by air embolism; N = sacrificed by suffocation in pure nitrogen. The figure followed by "h" indicates the period of time between the last injection of insulin and death in hours.

insulin; in a few instances they were apparent in the 2nd hour. Some rabbits failed to have convulsions in spite of very high doses of insulin given on several consecutive days; others were seized by most violent convulsions up to entire exhaustion following the administration of comparatively small doses. A spontaneous gradual recovery from the comatose or semicomatose state took place from the 6th or the 7th hour after the injection of insulin, even when convulsions were present, provided the insulin doses were

not excessively high for the particular rabbit, as they were on the 5th day for the rabbits of the 1st series.

All rabbits of the 1st series, except Rabbit 4, succumbed under large doses of about 20 units of insulin administered on the 5th

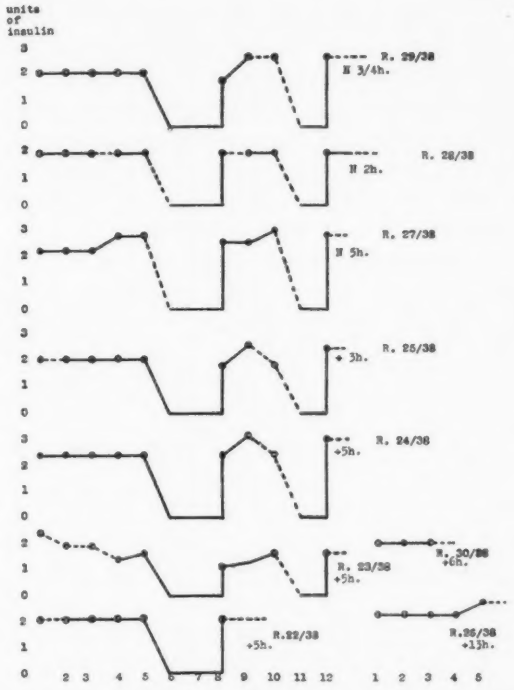

CHART 3. Effect of insulin (units per kilo body weight) on the rabbits of Series III. The broken lines indicate the days on which convulsions were observed.

+ = indicates spontaneous death; A = sacrificed by air embolism; N = sacrificed by suffocation in pure nitrogen. The figure followed by "h" indicates the period of time that elapsed between the last injection of insulin and death in hours.

day. They died between the 6th and the 12th hour following the insulin injection in spite of administration of glucose. Only Rabbit 4 survived when it was given glucose intravenously 9 hours after the insulin injection. It had been in deep coma with intermittent convulsions for more than 3 hours. Further subcutaneous

injections of glucose 3 hours later and on the next morning failed, however, to arouse it from the weak semicomatose condition in which it was, lying flat on its stomach, until it was sacrificed by air embolism 30 hours after the last injection of insulin.

Another remarkable reaction was shown by Rabbit 2 of the 1st series. On the 3rd day of treatment with insulin, 6½ hours following the injection of 5.8 units, it was seized by such violent convulsions that the respiration ceased. It was resuscitated by an intravenous injection of 10 cc. of 40 per cent glucose 1 minute later when the color of the blood in the ear artery had already turned deep blue. On the next day, however, the same dose of insulin was fatal after 3 hours, in spite of glucose given intravenously immediately after cessation of the respiration.

The great discrepancy between doses of insulin administered and reactions displayed was especially apparent in the 2nd series of animals. The 2 rabbits that were given the highest doses, from 4.4 to 8 and 9 units, repeatedly on consecutive days (Rabbits 17 and 18) failed entirely to have convulsions. In contrast, the initial dose of 4.4 units rendered Rabbit 14 deeply comatose 4 hours after the administration, and provoked frequent and violent convulsions during the next 1½ hours until glucose was given subcutaneously. This was, however, too late, and in spite of maintaining the blood sugar level above 100 mg. per cent by further injections of glucose, the rabbit was unable to raise its head or move its forelegs during the next 24 hours. Its condition became slowly worse so that after 48 hours the hind legs also were paralyzed. In that condition it was killed by nitrogen 55 hours after the 1st injection of insulin. Especially strange was the reaction of Rabbit 13 of this series. An initial dose of 5.1 units produced only slight listlessness on the 1st day. The blood sugar corresponded well to this slight reaction. It dropped from 106 mg. per cent to 56 mg. per cent 4½ hours after the insulin injection and rose spontaneously again to 113 mg. per cent 3 hours later. The rabbit was fed on the evening of the same day and was given glucose subcutaneously. On the next morning, however, the same dose of insulin was fatal after 4 hours.

In the 3rd series of rabbits in which the insulin doses were kept between 2 and 3 units, coma was not artificially interrupted by glucose except in Rabbit 23. In this rabbit the initial dose of 2.4

units was lowered to 1.2 and 1.6 units on the following days. Deep coma was usually terminated in this series by spontaneous recovery after a duration of about 3 hours. Rabbit 23, however, had to be given glucose subcutaneously on 4 days when its condition was so weak that spontaneous recovery could not be expected. The initial dose of 2.4 units, comparatively small for a non-fasting rabbit, had produced deep coma and convulsions from the 4th to the 7th hour after the injection. By this time when glucose was administered subcutaneously the rabbit had already suffered irreparable injuries to motor areas of the brain, for on the next morning it exhibited peculiar wiggling movements of its head and neck which never disappeared during the next 12 days.

Response of the Blood Sugar

The blood sugar level usually reached the lowest point in the 3rd hour after administration of insulin, then spontaneously rose again more or less rapidly. On the day when high doses of from 14.8 to 22.2 units were administered to the rabbits of the 1st series, the lowest blood sugar values were from 2.2 to 17.9 mg. per cent in the 3rd hour, even then rising spontaneously to from 25 to 37.5 mg. per cent in the 6th hour and from 45 to 71 mg. per cent in the 9th hour.

The height of the blood sugar level found in the rabbits corresponded well with the symtomatology exhibited. This, however, does not mean that the rabbits were always found in a state of deep coma or convulsions when the blood sugar level was at the lowest point. It was found that the blood sugar had to remain for some time at a low level before convulsions started. This level was about 40 mg. per cent. It was found that the incidence of convulsions depended much more on the failure of the organism to raise the blood sugar spontaneously again above a critical threshold within about 1 hour than on the deep fall itself. Thus, in the 1st series convulsions were not observed in the period when the blood sugar had almost entirely vanished, but in a period more than 1 hour later when it failed to rise again above 40 mg. per cent. On the other hand the rabbits, 17 and 18 for instance, that failed to have convulsions in spite of repeated injections of 8 and 9 units of insulin maintained a comparatively high blood sugar level. It was found to be 56 and 71 mg. per cent respec-

tively in these rabbits during the 4th hour after the injection of similar doses of insulin.

Changes in the Heart Musculature

Cross sections through the hearts of the rabbits of the first 3 series at close inspection showed a peculiarly mottled or checkered appearance of the wall especially distinct at the posterior wall of the left ventricle. Tiny dots, fine streaks and small flecks, pin-point to pin-head in size and gray in color, were recognizable there. The gross picture was somewhat suggestive of fatty infiltration although the color of the flecks was a bit too gray. Specific stains for fat with sudan III, scarlet red and Nile blue sulfate, however, were entirely negative and ruled out this suggestion. A peculiar hydropic swelling of the musculature was revealed histologically. In the areas involved the muscle cells stained much paler with hematoxylin-eosin than in normal areas. Individual muscle cells were swollen and enlarged, the striations were indistinct, and here and there had entirely vanished. The muscle cells appeared to be almost homogeneous and hyaline. The papillary muscles of the left ventricle, and occasionally of the right ventricle also, presented a similar appearance. In most of the hydropic muscle cells the nuclei were visible but were enlarged and pale. Swelling of the muscle cells was occasionally so marked that the blood capillaries were entirely compressed. In other areas of the same heart the capillaries of such hydropic foci were filled to capacity with erythrocytes giving the appearance of an artificially injected heart. This appearance, which indicated the capillaries had been incapable of constriction at the moment of death, was suggestive of stasis or severe local functional circulatory disturbance just prior to death. There was, however, no evidence that stasis might have been developed a considerable period of time (perhaps 1 hour or more) prior to death. In such an instance some massing of leukocytes would have been expected in the adjoining capillaries; this however, was not observed.

The glycogen stain (Best's method) was negative in these hydropic areas. In all the hearts of rabbits of the 4 series examined only very slight traces of glycogen, if any at all, were found histologically except in the cells of the conductive system which had preserved a glycogen content morphologically well demonstrable.

The hydropic foci in the musculature were the principal and constant finding in the hearts of practically all rabbits of the first 3 series, whether they died spontaneously following entire exhaustion or were sacrificed at various periods of the insulin shock by air embolism or suffocation in nitrogen. The size of the areas involved and the severity of the changes, however, varied considerably in the 3 series and in the single animals of a particular series. While in most animals the normal structure of the swollen muscle cells was preserved, in some instances small or even larger vacuoles were present in certain muscle cells. This was observed in Rabbit 1 of the 1st series, for instance, which on the 5th day of the experiment died spontaneously 5½ hours after the administration of 14.8 units of insulin. An autopsy was performed 15 minutes after death.

The hydropic changes in general appeared to be of quite recent formation, as if produced during the last exposure to an insulin shock. In the majority of cases there was no reactive cellular proliferation and no other sign indicative of previous cell destruction. Only 4 rabbits (1 and 2 of the 1st, and 22 and 23 of the 3rd series) showed evidence of irreversible damage to a few muscle cells of the many involved in hydropic changes. These cells had lost their nuclei, and their hyaline appearing and at times shrunken cytoplasm was invaded by scavenger cells, while a slight round cell infiltration was present in the interstitial spaces.

It was essential to rule out the fact that the method of sacrificing the rabbits was responsible for the formation of the above described hydropic changes. This was improbable from the beginning, for the rabbits that died spontaneously showed the same changes as those killed by air embolism or suffocation in a nitrogen chamber. The suffocation had to be given specific consideration, since the same changes could be produced by repeated anoxic shocks, as has been reported elsewhere. Five rabbits of the 4th series, which were sacrificed 7 weeks after the termination of the insulin treatment, served as controls in this respect. After they were placed in a nitrogen chamber, respiration and action of the heart ceased within 6 to 9 minutes. During this period the heart had been working under the strain of rapidly increasing anoxemia until it failed. But morphologically there was no trace of a hydropic swelling and vacuolization of cardiac musculature was

not observed. This indicates that a longer period is required for the development of these morphologically demonstrable changes. Many control rabbits sacrificed by air embolism after being used for various other purposes also failed to show these changes. On the other hand a single insulin shock of the duration of 4 to 5 hours was sufficient to produce these changes. Rabbit 14 is an example. The hydropic changes in its heart were very definite and widespread when it was sacrificed 55 hours later. Swelling and slight multiplication of the cells of the capillary walls in the areas involved made it doubtful whether or not the hydropic changes had surpassed the state of entire reversibility in this instance. Rabbit 13 showed definite hydropic changes but there was no evidence of irreversible damage. This rabbit died about 4 hours following the 2nd administration of 5.1 units of insulin. On the 1st day it was apparently not severely affected by that dose. The rabbits of the 3rd series which had been given daily doses not exceeding 3 units showed the same hydropic changes when they died or were killed from 2 to 5 hours after the last injection of insulin. Finally, Rabbit 33 of the 4th series, which 5 hours after the injection of a single dose of 0.82 units of insulin was exposed for 30 minutes to an anoxic shock and killed at the end of it, exhibited definite hydropic changes in the heart. The blood sugar dropped to 49 mg. per cent 2 hours after the insulin injection, and rose again to 69 mg. per cent immediately before the beginning of the anoxic shock.

In summary, it may be said that hydropic changes in the heart can be produced by a single dose of insulin large enough to produce a comatose or semicomatose condition for several hours. Convulsions were no prerequisite as shown by Rabbits 17 and 18, for instance, which had never had convulsions but exhibited quite definite hydropic changes in the heart.

Histological study of the hydropic changes in a single case did not permit a clear decision as to their reversibility, but this was possible by reviewing and comparing the entire series of animals. If the hydropic changes were the morphological equivalent of beginning irreversible cell destruction, evidence of fully developed necrosis, reactive cellular proliferation or scarring should have been found in the hearts of the rabbits that were repeatedly exposed to severe insulin shock, as were the rabbits of the 2nd and 3rd series. This, however, was not the case; in only the few

mentioned exceptions was there evidence of such a development. Even then, only a few cells of a comparatively large hydropic area were definitely necrotized and invaded by scavenger cells. Neither was there evidence of irreversible damage in the muscle cells that contained large vacuoles. These failed to give a positive reaction for fat or glycogen.

Of all the rabbits that were treated with smaller doses than those of the 1st series, only 22 and 23 of the 3rd series exhibited cellular reactive changes in the hydropic areas of the heart. They were the rabbits that had been most sensitive to insulin, as they were seized by violent convulsions after the administration of about 2 units of insulin on the 1st day of the experiment. They finally died in the 5th and 6th hour after the insulin injection on the 9th and 12th day, respectively, when the treatment was continued. In the heart of Rabbit 23 small foci of round cell infiltration were found centered around a necrotized muscle cell which was invaded by scavenger cells. This arrangement permitted their distinction from foci of chronic myocarditis which are rather common in the hearts of rabbits (Miller [3]). Fresh foci of hydropic swelling were also present. In Rabbit 22 there was found an entirely hyalinized area in the large papillary muscle bordering an especially severely involved hydropic area.

The absence of such changes in the majority of the rabbits showed that the hydropic changes on the whole can be regarded as entirely reversible. The complete absence of chronic inflammation or scarring at the usual site of the hydropic changes in the rabbits of the 4th series which were sacrificed after a period of recovery of 7 weeks strongly confirms this view.

As to the period of time required for the full restoration of the hydropic cardiac muscle cells, definite figures cannot be given. It may only be noted that Rabbit 36 of the 4th series showed scarcely a remnant of such changes when it was sacrificed by suffocation in pure nitrogen 2 days after the last insulin administration and 3 days after the last anoxic shock.

Changes in the Skeletal Musculature

Gross examination of the skeletal musculature was not suggestive of severe changes. There were no gross disruptions in any case, not even in the rabbits that had had the most violent convulsions for hours. Portions of the iliopsoas muscle, the muscu-

lature of the thigh, abdominal wall and diaphragm were routinely removed at autopsy and studied. Histologically, however, quite marked changes were found, producing a checkered or mottled appearance of the stained muscle sections, somewhat similar to the hydropic changes described in the cardiac musculature. Many fibers stained lightly, were swollen, and had partly lost their striations. In many rabbits of the first 3 series still more definite changes were present also, especially in the iliopsoas muscle. They consisted of partial or entire disruption of single muscle fibers. They were found with variable frequency at times in each field of low microscopic magnification; in other instances one or two broken fibers only were present in a whole section. Not infrequently a muscle fiber was broken into many short segments which were held in their position by the sarcolemma only. Here and there merely an infarction of a muscle cell had occurred; the reactive cellular infiltration, however, gave evidence of the intravital occurrence of the injury. All transitional stages from the most acute disruption to healing and organization of the necrotized segments were observed. In fresh material, as in Rabbit 33, which died 5½ hours after the 1st injection of insulin, the broken ends of a torn fiber were retracted, swollen, of a hyaline or waxlike appearance and were pale staining; a few erythrocytes were present at the ragged ends of the disrupted fibers. Larger hemorrhages, however, were not found in such areas. Even in fresh disruptions a massing of muscular nuclei was often noted near the ends of torn fibers. These, apparently, had been mechanically dislodged. At a later stage a slight infiltration of the broken fibers by leukocytes, and later by round cells was observed. In instances such as Rabbit 23, which had been seized by severe convulsions on the 1st day but had been kept alive 12 days, definite calcified segments of broken muscle fibers were found. In some instances single muscle cells definitely shrunken and infiltrated by round cells were found. Obviously an excessive hydropic swelling alone had been able to necrotize these cells.

In all probability the violent convulsions observed played a major part in these muscular changes. Slight fibrillar muscular twitchings that escaped the attention might also be a sufficient mechanical factor.

As to the glycogen content of these muscles, it can be stated

only that they were morphologically free of glycogen. Chemical tests were not performed.

Changes in the Liver

In the majority of the rabbits of the first 3 series the liver was definitely changed in gross appearance. It was somewhat enlarged, peculiarly stiff, and firmer in consistence than normal but not doughy, as in a fatty liver. The color was more grayish than normal and cross sections were frequently abnormally dry. On close examination with the naked eye a bright central area of the lobules could be distinguished from the darker, more brownish red periphery. The gross appearance was somewhat suggestive of central fatty infiltration although no fatty film could be observed on the knife used for making the cuts. Aside from these diffuse changes, in a few cases, as in Rabbits 4 and 14, irregularly distributed grayish foci, pin-point to pin-head in size, were found. Their whole appearance was quite suggestive of focal necrosis.

Histologically by specific staining the suggestion of fatty infiltration was entirely ruled out. Only in Rabbit 26 was a marked fatty infiltration of the liver found, but only in the periphery and not in the center of the lobules. The marked difference between periphery and center of the lobules, recognized in gross was, however, found histologically also. In contrast with a passively congested liver, the capillaries in the centers of the lobules were markedly narrower than at the periphery, as though compressed by the large and swollen hepatic cell strands present. A photograph of the liver of Rabbit 30 which died spontaneously may serve as illustration.

The main factor responsible for the pale staining of the centers of the lobules with the hematoxylin-eosin stain was the changed histological structure of the hepatic cells, which were enlarged, swollen and extremely vacuolized, frequently to such a degree that the entire cell seemed to be emptied. The nuclei of most of these definitely changed cells were comparatively little involved. They were in the center of the cell, were not pushed to the periphery or sickle-shaped, as in severe fatty infiltration, and usually preserved their normal density. Only relatively few cells had lost their nuclei or exhibited a shrunken or pyknotic appearance. The pale centers showed usually quite a sudden transition to the better

stained and more compact cell strands in the periphery. The pale appearance of the central cells was simply produced by a spreading out of the cellular cytoplasm over a wider area because of intracellular storage of a material that did not stain with hematoxylin.

In the first 2 series the pale areas of the lobules were usually confined to the central third or half of the lobule. In the 3rd series the pale areas extended more toward the periphery, and comprised almost the entire lobule with the exception of a small rim around the branches of the portal vein. Best's glycogen stain revealed that these vacuolized cells were laden to full capacity with glycogen, while the small compact cells at the periphery were morphologically entirely depleted.

The livers of the 5 rabbits of the 4th series which had been sacrificed by suffocation in nitrogen 7 weeks after termination of the insulin treatment, and the livers of 5 normal rabbits which were sacrificed by blows on the neck served as controls. In accordance with the findings of Arndt [4] and Villaret and coworkers [5] the control rabbits showed a rather even distribution of a moderate amount of glycogen throughout the entire lobules of the liver. In the livers of the rabbits killed by suffocation the glycogen was slightly diminished in the peripheral parts of the lobules.

Compared with these control rabbits the main difference found in the livers of the rabbits of the first 3 insulin series was the peculiar distribution of the glycogen present. In sharp contrast with the entire depletion of cells at the peripheries of the lobules, there was a definite overload of glycogen in the cells of the centers of the lobules. Based on histological estimation this overload was so marked that the total amount of glycogen in the liver appeared to be larger than in the control rabbits.

Even the rabbits of the 1st series, which on the last day had been given extremely high doses of insulin, still showed considerable glycogen in the centers of the liver lobules, in spite of violent seizures lasting for hours, and death from complete exhaustion. This was found even when the autopsy was delayed up to more than 1 hour after death. Only Rabbit 5 was an exception. It was given the highest dose of insulin, 22.2 units per kilo body weight. The blood sugar dropped to 17.9 mg. per cent 2 hours later, and was found to be 29.5 mg. per cent in a sample of blood withdrawn directly after the first seizure, 5½ hours after the injection of

insulin. After 4 hours of intermittent convulsions 5.2 gm. of glucose were injected intravenously and subcutaneously in 20 per cent solution. This terminated the muscular twitching within 30 minutes but could not save the rabbit. It was autopsied 2 hours after death. The entire glycogen content of the liver was depleted and the liver presented histologically the appearance of acute passive congestion, in contrast with the usual findings. The hydropic changes in the heart of this rabbit were especially marked in the walls of both ventricles.

Rabbits 3 and 6 of the 1st series which had not been given glucose on the last day of the experiment, although they were comatose for hours, also showed a marked storage of glycogen in the centers of the lobules of the liver when they died 7½ and 8½ hours, respectively, following injection of insulin.

The 4 rabbits (15, 16, 17 and 18) of the 2nd series constitute an interesting group. Their blood sugar content was determined immediately before they were sacrificed by air embolism on the 6th day of the experiment when they were treated with doses ranging from 6.6 to 9 units. Rabbits 15 and 16 had had convulsions on preceding days; Rabbits 17 and 18, however, had none. They were sacrificed about 5 hours following the last insulin injection. Rabbit 15 had just had the first convulsion on this day. The blood sugar level then was 22 mg. per cent, but the liver cells in the central half of the lobules were overladen with glycogen, the capillaries being constricted. The blood sugar of Rabbit 16 was 20 mg. per cent. It was weak and comatose, but had had no convulsions on the 6th day of the experiment. In the liver glycogen was present only in the central third of the lobules, the single cells containing not more than normally found throughout the lobule. The capillaries in the center were still more constricted than those at the periphery. Rabbits 17 and 18 were somewhat listless but by no means exhausted when they were sacrificed. Their blood sugars of 49 and 59 mg. per cent respectively corresponded well to their general response. Their livers resembled closely those of normal rabbits, the capillaries having about the same width throughout the lobule and some glycogen being present in all cells, somewhat more in the liver of Rabbit 17 than in that of 18.

Comparison of these 4 rabbits indicates that those able to re-

lease the glycogen storage in the liver gradually into the blood were able to maintain a fair blood sugar level and avoid convulsions, in spite of high doses of insulin. The other rabbits, in whose livers considerable amounts of glycogen were locked up, developed severe symptoms although their insulin doses were not so high.

The livers of all rabbits of the 3rd series, with the exception of Rabbits 26 and 30, were laden with an amount of glycogen scarcely ever found under normal conditions, although several of these rabbits died spontaneously of exhaustion during a shock period.

Rabbit 26 was found dead in the 13th hour after the injection of 2.6 units of insulin on the 5th day. It had had convulsions on this day beginning 2 hours after the injection of insulin and continuing intermittently for more than 5 hours. On the 4 preceding days its response to 2.2 units of insulin had been only weakness and listlessness with spontaneous recovery after several hours. During these days its blood sugar level did not drop below 60 mg. per cent during the 2nd and 3rd hour after the injection of insulin. Its liver showed a typical peripheral fatty infiltration, being histologically entirely depleted of glycogen. The capillaries were wider in the centers than at the peripheries of the lobules. This was the only rabbit that showed a fatty infiltration of the liver.

Rabbit 30, treated for 3 days with 2 units of insulin, did not show severe symptoms on the first 2 days, but on the 3rd day seizures began in the 5th hour after the injection of insulin and were repeated several times up to its death at the end of the 6th hour. On that day the blood sugar was 41 mg. per cent at the end of the 1st hour after the injection of insulin, and did not rise to more than 51 mg. per cent 3½ hours after the first seizure. The liver was histologically depleted of glycogen although it presented the reverse picture of a passively congested liver.

Obviously all carbohydrates available in the organisms of these 2 rabbits had been used up during days of treatment with insulin. This might be the reason that on the last day convulsions began comparatively early and the blood sugar failed to rise spontaneously again. It was all the more striking that the other 7 rabbits of this series which were treated with similar doses exhibited so much glycogen in the liver.

Thus, Rabbit 22 died on the 9th day of the experiment, 5 hours

after the injection of 2 units of insulin. The same dose had produced convulsions on the preceding day, from which it had recovered spontaneously. Although the autopsy was not performed until 1 hour after death, the entire liver was quite overladen with glycogen.

Rabbit 23 had been the most sensitive of all the animals to insulin. Doses of 1.6 to 2.4 units had produced severe coma and seizures during 12 days. Its blood sugar would fall to a level between 22 and 33 mg. per cent during the period from the 2nd to the 5th hour after the injection of insulin. When it finally died, 5½ hours after the last injection of 1.6 units of insulin, its liver, removed 20 minutes after death, was heavily laden with glycogen. The changes in the vital organs, such as the heart and the brain, were in contrast exceptionally severe, practically all ganglion cells of the brain cortex, for instance, having disappeared.

Additional evidence that the livers of rabbits may store and lock up large amounts of glycogen under the influence or, more cautiously speaking, after the administration of insulin, while the whole organism may perish for lack of glycogen, is shown by the next group of rabbits to be described.

Rabbit 25 died spontaneously on the 12th day of the experiment exactly 3 hours after the injection of 2.5 units of insulin. When the respiration ceased no attempt at artificial respiration was made. A blood sample withdrawn from the hepatic vein 10 minutes later had a sugar level of 476 mg. per cent, evidence that considerable glycogen available in the liver was converted into blood sugar after cessation of the respiration due to the effect of anoxemia. The blood sugar level before the insulin injection had been 116 mg. per cent on this day. It would fall to from 27 to 33 mg. per cent during the period from the 2nd to the 5th hour after the injection of the same dose of insulin on several previous days. Histologically this liver was also overladen with glycogen.

Rabbits 28 and 29 were sacrificed so that the glycogen content of the liver could be studied in the 1st hours after the injection of insulin. Rabbit 29 had had severe convulsions on the previous day, the 12th day of the experiment, but had recovered spontaneously. On the next morning its blood sugar was 168 mg. per cent. Forty minutes after the injection of 2.7 units of insulin it dropped to 79 mg. per cent. Immediately after the withdrawal of

this blood sample this rabbit was suffocated in a nitrogen chamber. Thirteen minutes after the onset of anoxemia a blood sample withdrawn from the hepatic vein contained 357 mg. per cent of sugar. The blood sugar of Rabbit 28 was similarly high, 155 mg. per cent, on the same morning. It dropped to 62 mg. per cent 2 hours after the injection of 2 units of insulin. A short convulsion of about 1 minute's duration occurred immediately after the withdrawal of this sample of blood. The rabbit was then also suffocated by nitrogen. Six minutes after the onset of fatal anoxemia a blood sample withdrawn from the hepatic vein contained 116 mg. per cent of sugar. Histologically the livers of these 2 rabbits were still overladen with glycogen, as shown by Figures 18 and 19.

That the rabbits of the 4th series which had been given doses of about 1 unit reacted in a similar way is shown by the following results. Two hours after a single dose of 0.8 units the blood sugar of Rabbit 33 dropped to 49 mg. per cent and increased again to 69 mg. per cent after 3 hours. The rabbit was then exposed for 30 minutes to an anoxic shock that was fatal. Fifteen minutes after death blood from the hepatic vein contained 500 mg. per cent of sugar. Histologically the entire lobules of the liver were markedly overladen with glycogen. Three other rabbits (31, 32 and 34) which were treated the same way, except that the anoxic shock was not fatal, showed the following movement of the blood sugar. It was, on the average, 63 mg. per cent 2 hours after the insulin injection, 99 mg. per cent at the 5 hour mark, and 159 mg. per cent ½ hour later after the termination of the anoxic shock.

In the central areas of the lobules that were definitely overladen with glycogen relatively little morphological evidence was found indicating irreversible damage to the hepatic cells. Only a few cells had lost the nuclei or exhibited a pyknotic appearance. It could therefore be expected that the liver cells on the whole might be able under proper conditions to rid themselves of the overload of glycogen and return to a normal state. However, in the animals that had been treated with excessively high doses of insulin, such as the rabbits of the 1st series on the last day, there was found definite cellular damage. These rabbits exhibited single cells or small groups of cells in the liver with an eosinophilic cytoplasm and shrunken, hyperchromatic or even pyknotic nuclei. It would have been difficult to differentiate this appearance from postmor-

tem changes if it had not been for the presence of definite areas of focal necrosis in the livers of half the rabbits of the 1st series. These areas of necrosis were usually located in the centers of the lobules extending more to the periphery than to the center, occupying a sixth to a third of a lobule. Judged by the leukocytic and cellular reaction in the vicinity, they apparently formed on the last day of the experiment when high doses of insulin were given. In Best's stain for glycogen the necrotic foci were seen to be depleted of glycogen, even when all neighboring cells were laden. In the 2nd series only Rabbit 14 showed focal necroses in the liver. In accord with its survival for 55 hours after the single insulin dose given, the leukocytic and cellular reaction in the vicinity was marked. Many bizarre, hyperchromatic giant nuclei were found in the liver of Rabbit 13 which died about 4 hours after the second injection of 5.1 units of insulin. In only 1 rabbit of the 3rd series (Rabbit 30) were small foci of quite recent necrosis found. In spite of relatively low doses of insulin the liver of the rabbit was entirely depleted of glycogen. The animal died after violent seizures 6 hours after the last injection of insulin.

In a few instances a slight round cell infiltration, non-leukocytic in character, was noted around the small branches of the portal vein of 1 rabbit of the 1st series, 2 of the 2nd, and 3 of the 3rd. None of the rabbits of the 4th series showed such changes. It is hard to decide whether or not these changes are sequellae of the insulin treatment. Chaikoff, Connor and Biskind [6] reported cirrhosis of the liver in dogs that had been kept alive for years by injections of insulin after extirpation of the pancreas. Possibly changes similar to these are forerunners of such a development.

Discussion

In the experiments reported here, changes in the brains of the rabbits were so marked and dominant that spontaneous death when recorded could be attributed to their occurrence. According to the studies of Himwich and coworkers [7] these changes can be attributed readily to starvation of the brain, which maintains its metabolism solely by combustion of carbohydrates. Our studies have shown that the heart and skeletal musculature also are severely affected when the blood sugar drops to and is maintained

at low levels for hours as in insulin shock. Müller [8] has described electrocardiographic changes in patients during insulin shock treatment for schizophrenia. These changes were similar to those known to be present in severe organic myocardial conditions, but were transient and disappeared after termination of the insulin shock. Salm [9] described attacks of cardiac weakness and failure in such patients. Even if these might be considered sequellae of damage suffered by circulatory centers in the medulla rather than direct effects of the low blood sugar level on the heart musculature, observations reported by Larsen [10] furnish evidence for such a direct effect. He found electrographic changes similar to those reported by Müller when he subjected diabetic patients who were merely under the influence of small therapeutic insulin doses to the anoxic test designed for the objective evaluation of the actual power of the heart. The same patients showed normal electrocardiograms during the anoxic test when examined without insulin injections.

The hydropic changes reported above in the heart musculature of rabbits treated with insulin can be regarded as the anatomical equivalent of these electrocardiographic changes. They are also reversible, on the whole, and only in a few exceptional cases did small muscular necroses occur.

Weakening of the heart by insulin treatment is shown by another observation also. Normal rabbits sacrificed by confinement in a nitrogen chamber died within 6 to 9 minutes. Their lungs collapsed when the thorax was opened. Rabbits that had been in insulin shock for a few hours, even when no convulsions occurred, developed a marked pulmonary edema with bloody foam at the mouth when sacrificed in the same way. A weakened action of the left ventricle was obviously its main cause. Goldblatt's [11, 12] observation that rabbits with insulin hypoglycemia died of strychnine convulsions surprisingly rapidly can be similarly interpreted.

In the skeletal musculature still more severe changes were found than those in the heart. They were equivalent to the early and marked muscular weakness the rabbits displayed during insulin shock. These changes were present even when no convulsions had occurred. Various authors have described the vanishing of the glycogen content of the muscle after the onset of insulin convulsions (Baur, Kuhn and Wacker,[13] Dudley and Marrian,[14] Hoet

and Marks [15]). Hetényi [16] and Peyer [17] found that when the glycogen in the cardiac muscle had dropped during insulin convulsions from 0.35 to 0.12 per cent, that of the skeletal muscle was 0.05 per cent and less.

The anatomical findings in the heart and skeletal muscle seem to indicate that the heart as well as the skeletal musculature cannot act in the living animal for periods extending over several hours without structural damage unless a certain high level of blood sugar is maintained. This conclusion seems not quite to concur with observations reported recently by Himwich and Fazekas.[18] They found that the oxygen consumption of muscle did not diminish like that of brain when the glucose concentration of the blood was lowered by insulin administration. This led them to the conclusion that muscle is able to maintain its energy metabolism solely by the combustion of fat and that carbohydrates are not essential. Our anatomical findings at least make it clear that muscle in an insulinized animal requires carbohydrates in addition to the oxidative metabolism which might be maintained solely by fat. Without venturing a suggestion concerning the phase of the complicated muscular metabolism for which glycogen is indispensable, it might be conjectured that at times, for short periods, during action the oxidative metabolism is unable to provide momentarily the energy required so that the muscle has to rely on sources of energy that are accessible anaerobically. In all probability it is this type of energy metabolism for which glycogen is required. This conclusion finds some confirmation in observations made by Bogue, Evans and Gregory.[19] They found that the storage of glycogen in the beating heart which was artificially perfused was exhausted in about 3 hours. A sudden failure occurred at the point of this exhaustion which could be prevented only by supplying glucose with the perfusing fluid. They also observed that a heart excised after its depletion of glycogen fell immediately into rigor mortis, *i.e.* underwent irreversible changes. Concurring with these observations are those of Bayliss, Müller and Starling.[20] They maintained the action of artificially perfused hearts for several hours longer when the percentage of glucose in the perfusing fluid was increased.

The changes found anatomically in the heart and musculature were unevenly distributed. This undoubtedly points to a vascular

influence. Under normal conditions it might be insignificant whether a muscle fiber gets its blood supply mainly from a capillary that arises directly from an arteriole, or from a net capillary that arises from another capillary. But when the nutritional qualities of the flowing blood are lowered as much as in insulin shock, this might become a factor of importance, especially when strenuous action of a muscle is required. Even the difference of anatomical relationship to the venous or arterial end of a capillary might be of weight under such conditions. By the close interrelationship of capillary wall and tissue pathological metabolic products that are produced by a muscle fiber inadequately supplied are likely to affect the wall of the capillary and produce local circulatory disturbances up to entire stasis. This would open a vicious circle that might finally be the cause of irreversible cellular changes.

Another actual observation has to be considered in this connection. During insulin convulsions and often even 30 minutes after the termination of severe seizures, the ear arteries of the rabbits became definitely constricted, frequently so intensively that it was impossible to obtain blood from the ear veins even when xylol was applied locally. If such vascular constrictions also occur in the parenchymatous organs and in the musculature, they might definitely contribute to severe tissue damage.

On the basis of studies by Dudley and Marrian,[14] McCormick and Macleod,[21] Staub,[22] Bissinger, Lesser and Zipf,[23] Cori,[24] Barbour *et al.*,[25] and many others, it is generally accepted that in the normal animal the glycogen content of the liver is depleted by insulin given in doses that produce convulsions. In a survey of the literature Staub [22] stated that in normal animals it is impossible to obtain an increase in the glycogen content of the liver by insulin unless, as in fasted animals, a deficit of that hormone is brought just to an equilibrium. Any excess would produce a decrease.

There seems to be a definite discrepancy between these statements and the results obtained in our experiments. In order to understand it several factors have to be considered. First, the method of our experiment was not that usually employed, in so far as no fasted normal animals were employed which, following a single injection of insulin with simultaneous feeding of glucose, or without any feeding, were sacrificed after a short period of a few hours.

Our rabbits could be considered normal only at the time they received their 1st injection of insulin. The 2nd injection given 24 hours later was administered to a rabbit that was already in a state of recovery from a serious disturbance of its carbohydrate metabolism. Each subsequent injection of insulin may have met with a somewhat different constellation of the hormonal equilibrium and its effect on the organs it governed. Some evidence for this contention is given by the blood sugar level which in the 2nd week of the treatment of the 3rd series of rabbits was found at times above 150 mg. per cent in the morning prior to the administration of insulin. The different states in which the rabbits received insulin after the 1st day may therefore be answerable mainly for the differences in our results from those generally reported. Another reason may be that in our studies the staining method of Best was employed for ascertaining the glycogen content of the organs. This method does not show glycogen in as small amounts as are chemically still demonstrable, but it showed the peculiarities of its distribution in the liver and the concomitant vascular effects that might be worth while following still further.

The results of the experiments reported here indicate that the fate of a rabbit exposed to insulin in shock dosage depends very much on the reaction of its liver. Two types of reaction were observed. In rabbits that endured insulin doses of 8 and 9 units per kilo body weight repeatedly administered without severe symptoms, and that maintained a blood sugar level above 50 mg. per cent, the liver contained only a slight amount of glycogen almost evenly distributed with little preference for the centers of the lobules. The capillaries were about the same width throughout the lobule. Obviously such a liver was able to release all glycogen synthetized during the shock period as blood sugar, preventing severe starvation of the brain and convulsions thereby. In rabbits with this type of reaction a total exhaustion of glycogen present in the body might still occur. Thus, rabbits that had endured large insulin doses on the preceding days finally died, at times surprisingly, when the dose was only a little increased or just merely repeated. In such instances the liver was regularly found to be free of glycogen.

On the other hand, rabbits that had displayed severe symptoms — convulsions for hours following the administration of even

comparatively small doses of insulin — and whose blood sugar had dropped to vanishing levels, showed a liver overladen with glycogen when they died or were sacrificed at various periods of the shock. The capillaries in the liver, especially in the central portions of the lobules, which were mainly overladen with glycogen, were markedly constricted, producing the impression that thereby the glycogen was locked up. The majority of the rabbits showed this type of liver.

The influence of the dose of insulin was demonstrable in so far as the glycogen storage in the livers of rabbits given doses between 2 and 3 units was usually larger than in those receiving 5 to 20 units per kilo body weight. In principle, however, both types of reaction were found in all series of rabbits.

Only a few experimental observations are reported in the literature which concur with the results reported above. Frank, Nothmann and Hartmann [26] found an increase in the glycogen content of the liver in fasting rabbits when small doses of insulin were given. The experiments most important in this respect are those of Goldblatt.[11, 12] He used young rabbits (about 400 gm. in weight) which had been fasted 24 hours prior to the experiment. The glycogen content of the liver increased up to sevenfold when insulin in convulsion-producing doses was given, and it was not depleted when the rabbits finally died of exhaustion. The actual observations of Goldblatt were confirmed by Corkill.[27] Other species, such as mice, ferrets and chickens, however, did not accumulate glycogen in the liver under the same conditions. Corkill came to the conclusion that the increase of glycogen in the livers of rabbits was not an effect of insulin alone but a complicated response of the organism possibly produced by the reactive output of adrenalin. Cori [24] had shown that adrenalin is able to produce hyperglycemia without depletion of the glycogen in the liver. Goldblatt had found that adrenalin did not prevent the increase of glycogen in the livers of his insulin rabbits, and in specific experiments Corkill showed that adrenalin increased the glycogen in the liver about four to five times, as compared with the average content of fasted control rabbits. In Ricker's laboratory, Loeffler and Nordmann,[28] by direct microscopic observation of the liver, found after injection of insulin in shock dosage a definite vascular constriction present for 2 to 3

hours. During this period glycogen was stored in the livers of rabbits, while in rats under the same conditions no glycogen but fat appeared, accompanied by a still more extensive vascular constriction. Insulin administered when a large storage of glycogen in the liver had been produced by an appropriate diet had a dilatory effect on the blood vessels at the peripheries of the liver lobules, simultaneously depleting the glycogen in these areas while the vascular bed and the glycogen in the centers of the lobules were not affected.

We are inclined to consider the vascular constriction in the liver a secondary effect of the insulin injected, or due to hormones secreted by the adrenal gland and the anterior or posterior lobes of the pituitary which reactively might be poured out into the blood stream. Hormones from these glands have been shown in recent years [29–31] to be effective in directing the carbohydrate metabolism.

At this stage it seems appropriate to remark that insulin administered in doses high enough to produce an initial marked hypoglycemia initiates a sequence of disturbances of the carbohydrate metabolism during which, obviously, hormones of antagonistic effects are liberated. These play their part in the carbohydrate metabolism, and produce vascular disturbances also.

Summary and Conclusions

Insulin in shock doses repeatedly administered to rabbits produced definite hydropic changes in the cardiac and skeletal musculature even when no convulsions were elicited. The posterior wall of the left ventricle and its papillary muscle were the most favored locations. The changes in the heart, on the whole, were reversible; only in exceptional cases did they progress to necrosis of small patches of cardiac muscle cells. In the skeletal musculature, especially in the iliopsoas and the thigh musculature, irreversible changes were more frequent. They were present as broken single muscle fibers in all stages of the process beginning with fresh breaks up to entire healing or calcification.

These changes are considered evidence of the necessity of carbohydrates for the working muscles in the body. They are in all probability indispensable for certain phases of the anaerobic metabolism.

The sensitivity of the individual rabbit to insulin varied widely. It depended rather on the response of the liver than on the dosages employed.

Two types of liver reaction to insulin were observed widely independent of the size of the doses. (1) The liver was able to release gradually all glycogen synthetized before and after the insulin administration from various sources (absorption from the intestines, lactic acid from the musculature). In such instances, which constituted a small minority, a comparatively fair blood sugar level was maintained and convulsions and severe damage to vital organs, such as the brain and the heart, were avoided for relatively long periods of time in spite of repeated administration of high doses of insulin on consecutive days. The rabbits died finally when all glycogen available was exhausted.

(2) The liver retained the carbohydrate available as glycogen and locked it up, particularly in the centers of the lobules which were definitely overladen. This response was accompanied by a marked constriction of the capillaries in the overladen areas of the lobules. Thus, while large amounts of glycogen were stored in the liver, the blood sugar dropped to vanishing levels and convulsions with severe damage to the brain and heart occurred. The rabbits that were especially sensitive to insulin showed this result in the liver. The influence of dosage on the type of reaction in the liver was insignificant when doses between 2 and 20 units per kilo body weight were administered, the smaller doses producing merely further extension of the glycogen-storing area toward the peripheries of the lobules.

The glycogen storage in the liver is considered to be the effect of insulin and various other hormones of antagonistic or synergistic effect on the carbohydrate metabolism produced by the adrenal gland and the anterior and posterior lobes of the pituitary. Simultaneous vascular effects of these hormones are considered essential to produce such changes.

Aside from the reactions described, small areas of focal necrosis in the liver were observed, especially in the series of rabbits treated with excessively high doses of insulin.

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DESCRIPTION OF PLATES

PLATE 6

FIG. 1. Section from the heart of Rabbit 1 showing a focus of edema around an artery with round cell infiltration.

FIG. 2. Section from another area of the heart of Rabbit 1 showing swelling and acute hydropic degeneration of the cardiac muscle cells.

FIG. 3. Higher power of section from the same heart shown in Figure 2.

FIG. 4. Heart from Rabbit 23. Necrosis of single cardiac muscle cells with round cell infiltration is seen.

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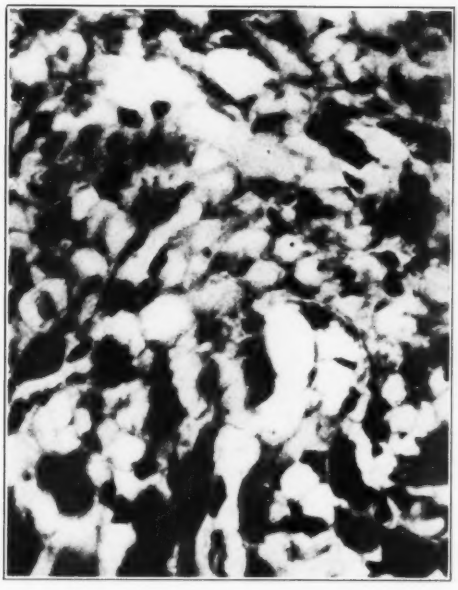

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Tannenberg

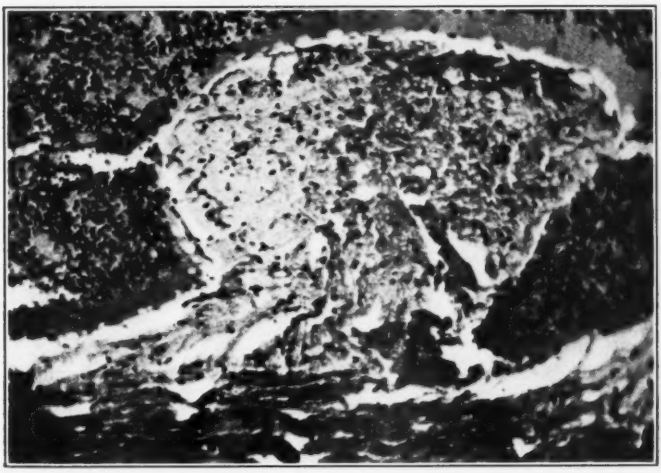
Changes in Rabbits Treated with Insulin

PLATE 7

FIG. 5. Section from the right ventricle of the heart of Rabbit 5 showing marked hydropic swelling of the inner layers of the ventricular wall.

FIG. 6. Section from the left ventricle of the same heart showing similar changes.

FIG. 7. Iliopsoas muscle from Rabbit 15. Breaks in single muscle fibers and reactive cellular proliferation is seen. For higher power see Figure 10.



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Tannenberg Changes in Rabbits Treated with Insulin

PLATE 8

FIG. 8. Heart from Rabbit 22 showing definite hydropic swelling and hyaline degeneration.

FIG. 9. Another area from the heart of Rabbit 22 showing hydropic swelling and hyaline degeneration.

FIG. 10. Higher power of field from the iliopsoas muscle of Rabbit 15 shown in Figure 7.

FIG. 11. Section from the liver of Rabbit 18 stained with Best's method for glycogen. Note the slight amount of glycogen present and the dilated capillaries.

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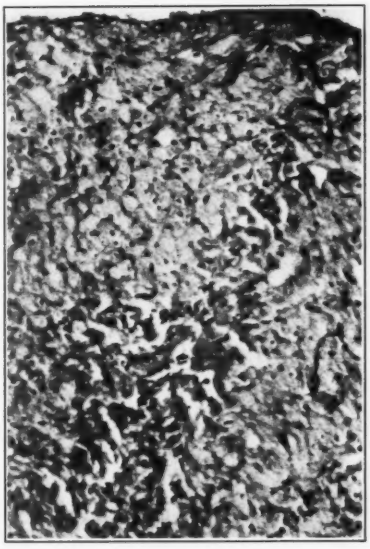

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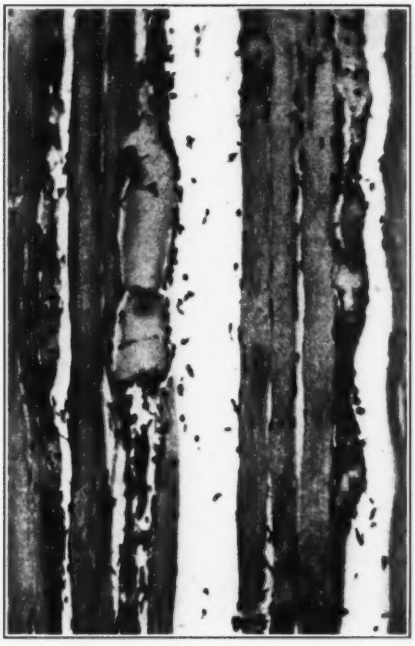

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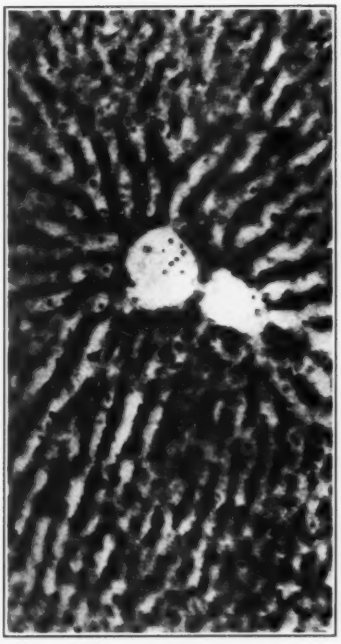

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Changes in Rabbits Treated with Insulin

PLATE 9

FIG. 12. Liver from Rabbit 1 stained with Best's glycogen method and showing a definite storage of glycogen in the central third of a lobule with depletion at the periphery. This rabbit was injected with 14.8 units of insulin on the last day of treatment.

FIG. 13. Higher power of section of the liver from Rabbit 1 shown in Figure 12 and stained by the same method.

FIG. 14. Liver from Rabbit 15 stained with Best's method for glycogen. A definite overload of glycogen is seen in the central halves of the lobules.

FIG. 15. Section of liver from Rabbit 23. The glycogen stain reveals a marked overload of glycogen in the entire lobule, especially in the center. This rabbit died from complete exhaustion after hours of convulsions.

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Tannenberg

Changes in Rabbits Treated with Insulin

PLATE 10

FIG. 16. Liver from Rabbit 30. Note the marked congestion of the capillaries around the branches of the portal vein and in the periphery of the lobules, and the vascular constriction in the centers of the lobules.

FIG. 17. Higher power of the same liver shown in Figure 16.

FIG. 18. Section of liver from Rabbit 29. Best's stain for glycogen shows the definite overload of glycogen present, especially in the center of the lobule. The rabbit was killed 45 minutes after the last insulin injection.

FIG. 19. Liver from Rabbit 28. The glycogen stain shows almost the entire lobule filled with glycogen. The rabbit was killed 2 hours after the last injection of insulin.

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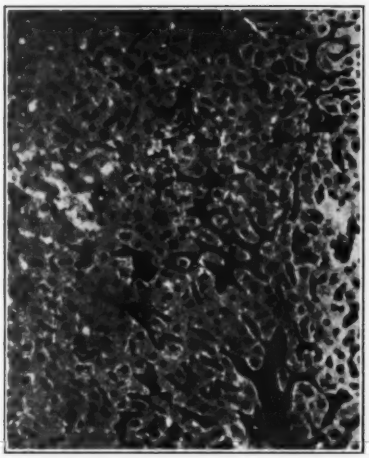

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Changes in Rabbits Treated with Insulin

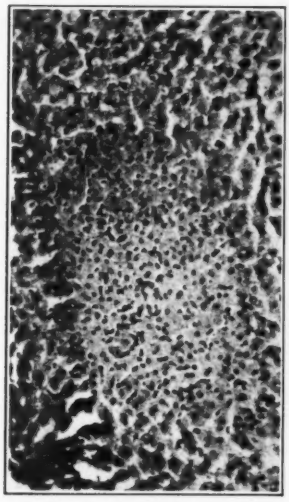
Plate 11

Fig. 20. Liver from Rabbit 4 showing focal necrosis of quite recent origin.

Fig. 21. Section of liver from Rabbit 14 showing similar changes.

Fig. 22. Section of liver from Rabbit 3 stained by Best's glycogen method showing necrotic hepatic cells depleted of glycogen.

Fig. 23. Section of liver from Rabbit 6 showing single cells with pyknotic nuclei and eosinophilic cytoplasm.



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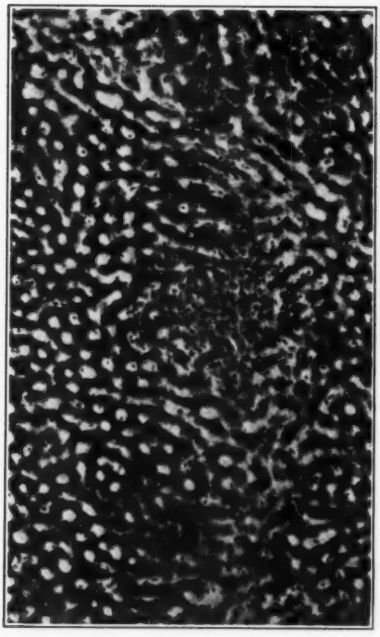

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23

Changes in Rabbits Treated with Insulin

GROWTH PROCESSES IN CARTILAGE AND BONE SUBSEQUENT TO GONADECTOMY AND ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT OF CATTLE IN IMMATURE MALE AND FEMALE GUINEA PIGS *

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Differences in the body growth of male and female rodents under normal and pathological conditions have been established.[1-3] This and the fact that in man the disposition toward diseases of the osseous system varies in accordance with the sex suggest the possibility that differences might also exist in the response of the cartilage and bone-forming tissues of the two sexes toward stimuli of various kinds. In former investigations (1935)[4] we have shown a growth-promoting effect of acid extract of anterior pituitary of cattle on the cartilage of immature guinea pigs, regardless of their sex. Subsequently similar observations were made by Zuck[5] (1938) in guinea pigs, and by Freud and Levie[6] (1938) in rats. In continuation of our studies[7] we found an increased hyperplastic and hypertrophic growth of the epiphyseal cartilage after ovariectomy alone, and in combination with anterior pituitary extract. Since then we have extended our studies to the condition of the joints, bone marrow, cortex of the long bones, ribs and vertebrae in ovariectomized immature guinea pigs.

Recently we performed in addition experiments on immature male animals. In some, the influence of castration on the growth of cartilage and bone was studied, and in others the interaction of castration and anterior pituitary was investigated and compared with the results obtained in ovariectomized animals under the corresponding conditions.

MATERIAL AND METHODS

In 43 male guinea pigs, born in the fall and winter, and weighing 135 to 160 gm., both testes were removed under ether anes-

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thesia by incision into the scrotum. Twenty animals did not receive any further treatment and were sacrificed after periods of 7, 9, 14, 21, 28 and 35 days. Fifteen guinea pigs received daily intraperitoneal injections of 1 cc. of freshly prepared acid extract of anterior pituitary of cattle. The injections were started immediately after castration. These animals were killed after periods of 7, 14, 21 and 28 days. In the third group, consisting of 8 castrated guinea pigs, the treatment with the extract was begun after 7, 14, 21 and 28 days respectively had elapsed following the operation. In these series, 7 injections were given and the animals were killed in groups of 2, 1 day after the last injection. Ten non-castrated animals of the same weight received a corresponding number of injections of acid extract, while 8 animals served as normal controls.

At autopsy the thyroid glands, adrenals, and in control animals one testicle also, were fixed in Petrunkevitch's solution and embedded in paraffin. The tibia from one side was taken out separately, while the knee joint of the other side was left intact by removing the tibia and femur as a whole. In addition, 2 to 4 ribs were removed in such a way that about 0.5 cm. of each cartilaginous and osseous part were preserved. Parts of the spine, comprising lower thoracic and upper lumbar vertebrae, were likewise removed. After the bones had become soft enough for cutting, the distal parts of the tibiae and the proximal ends of the femurs were cut off, and a sagittal section was made through the middle of the knee; the single tibia was cut in frontal sections. Thus, a study of the epiphyseal zone in two different planes was made possible. All bones were fixed, embedded and stained as described in previous papers.[8]

In addition, the joints, ribs and vertebrae of the 30 female animals which had served for the analysis of the endochondral ossification in ovariectomized guinea pigs [7] were examined. Altogether, the material of 91 guinea pigs was studied.

Although our investigations were not primarily concerned with the behavior of the weights of the animals under these experimental conditions, we thought it of interest to consider this factor also, and each animal was weighed three times a week.

In order to determine more definitely the rate of hyperplasia and hypertrophy of the cartilage cells of the epiphyseal line and

their mutual proportions, counts were made of the cells lying in certain rows. A mean was established by counting three rows in each section. Care was taken to choose layers in corresponding positions.

Observations

The mean weights of the animals are represented in Tables I and II.

Table I

Male Guinea Pigs

Experiment	Number of animals	Initial weight	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Normal	8	143	166	185	211	232	260
Injected	10	149	145	157	182	205	..
Castrated	28 *	147	173	200	226	254	315
Castrated and injected	15	149	147	162	194	220	..

* This figure includes 20 castrated animals and 8 additional castrated animals in which the injections were started after 1, 2, 3 and 4 weeks following castration respectively.

Table II

Female Guinea Pigs

Experiment	Number of animals	Initial weight	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Normal	13 *	140	165	195	213	242	272
Injected	13 *	143	149	157	173	197	238
Ovariectomized	19 *	145	165	195	230	252	280
Ovariectomized and injected	22 *	146	153	166	180	191	223

* In these figures are included 37 animals from experiments which have been conducted since our previous communication.[7]

Microscopic Examination

I. Normal Control Animals:

In both sexes the finer structure of the cartilaginous and bony tissues is the same. No differences could be detected. A detailed description has been given previously by us.[8]

II. Injected Control Animals:

Epiphyseal Line and Chondrophyte: Seven to 28 injections of the extract, 1 injection being given daily, produce typical changes

which are essentially alike in both sexes. These changes have been discussed in previous reports.[4]

Joints: Arthropathic lesions [9] may be found in both sexes. Loops of capillaries of the bone marrow advance into the cartilaginous pressure zone eroding the thin bony lamella which usually separates the calcifying cartilage cells from the bone marrow. In the *female* hypertrophic changes of the cartilage predominate with a tendency toward degeneration. In the *male*, although hypertrophy may be present at later stages, hyperplasia is more accentuated.

The *bone marrow* adjacent to the zone of ossification assumes a fibrous character.

Cortex: The appositional growth and the resorptive processes are stimulated but well balanced. Osteoid seams cannot be detected.

Ribs: After 7 injections, in both sexes, but more markedly in males, a degeneration of the chondromucoid ground substance and of the mature euhyaline cartilage cells becomes noticeable (Fig. 1). The cell nuclei become pyknotic and lumpy, and the cytoplasm becomes enlarged, vacuolated and disintegrated. At the chondro-osseous junction the resting and columnar cartilage cells proliferate and hypertrophy. These changes increase with increasing numbers of injections of acid extract. They have to be considered as precursors of the "acromegalic rosary" which we have described previously.[10]

Vertebrae: The zone of endochondral ossification shows a hyperplastic and hypertrophic growth of the resting and columnar cartilage cells and an increased tendency to calcification. In addition, osseous plugs ("Ossificationsluecken," Erdheim,[11] and Schmorl [12]) may appear. These plugs consist of osseous masses within the zone of columnar cartilage and result from degenerations within the cartilage; they contain transverse cracks and irregular cavities. Even after 7 injections, small sized plugs may be observed, and they may be essential in counteracting a possibly widespread spontaneous fracture of the vertebrae softened by degenerative processes. The cracks indicate possibly that tension differentials in the tissues exist which otherwise would lead to fractures. It is a striking fact that these plugs are not found within the epiphyseal line of the long bones but only in the verte-

brae. Erdheim [11] explains this occurrence by differences in the gross structure of these two types of bones. In the long bones the epiphyseal disc is firmly fixed, supported and protected against breakdown by the overlapping bony cortex. This latter condition is lacking in the vertebrae and the necessary support of the bones is supplied by the osseous plugs.

III. Gonadectomized Animals:

Epiphyseal Line: In *male* animals, 1 week after castration, the zone of endochondral ossification is of medium width and is patent. The chondromucoid ground substance which takes on a distinctly basophilic stain with hematoxylin is swollen, vacuolated and, in some cases, disintegrated. The network of the fibrils within the matrix is loosened and partly degenerated. Associated with these lesions are changes within the cartilage cells which may be summarized as follows: The cells of the resting and columnar cartilage become more numerous and the cytoplasm of the vesicular cartilage increases in size. Thus a typical hyperplasia and hypertrophy of the individual cells result. These phenomena were observed in all cases. In addition we detected occasionally very slight retrogressive changes within the cartilaginous cell rows similar to but less significant than those we reported after implantations of one-quarter of a gland of cattle anterior pituitary in young female guinea pigs on 2 consecutive days.[8] Increasingly from one week to another following castration the epiphyseal line becomes somewhat wider on account of an increased hyperplasia of the cartilage cells rather than because of an enhanced hypertrophy. The retrogressive changes within the intercartilaginous stroma are likewise augmented. After 4 to 5 weeks the epiphyseal line is very wide. At this stage degeneration of entire cartilaginous cell rows is seen. The cells of the hypertrophic calcifying cartilage zone are slightly increased in number and hypertrophic. At the periphery of these cells masses of calcium and lime salts have been deposited. The intercartilaginous matrix is scarce. The usually regular border line between the zone of calcifying cartilage and the bone marrow is preserved to a great extent. In some places, however, the breakdown and the replacement of the hypertrophic cartilage cells by bone have not at all, or only incompletely, been accomplished. Thus, in the majority of cases strands of densely

calcified hypertrophied cartilage are visible within the bone marrow. These thick strands which may contain as many as 5 or 6 or more cartilage cells show a longitudinal arrangement; the contours of trabeculae are already suggested. One has thus to deal with an increased calcification but incomplete ossification. The cartilaginous strands are accompanied by fibrous connective tissue and are surrounded by osteoblasts.

These findings are in contrast with the corresponding conditions in *ovariectomized* animals. In the latter, only a slight swelling and perhaps a trace of degeneration may be noticeable. There is an increased tendency to hypertrophy on the part of the cartilage

TABLE III

Ratio of the Number of Hypertrophic Cartilage Cells to the Number of the Proliferating Columnar Cartilage Cells

Experiment	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
Normal males and females	4–5:11	4:10	4:11	4:10–11	4:10
Castrated males	5:16	5:17	5:17–18	5:20	4:18–19
Castrated females	5:12	6:17–18	6:18–19	5–6:18	..

cells, while the rate of proliferation is about the same or slightly lower as compared with the males. On the whole, the epiphyseal line is wider in females than in males in the corresponding stages, the width in this case being determined by the degree of hypertrophy rather than that of hyperplasia. The tendency to calcification and ossification is less pronounced in the females. No strands of calcified cartilage within the bone marrow were observed, and ossification apparently proceeds at a well balanced pace, as indicated by a sharply demarcated regular boundary between the zone of calcification and the bone marrow.

A differential count of the number of cells in the columns of the proliferating cartilage, on the one hand, and the hypertrophic cartilage on the other, is given in Table III.

The data concerning the normal animals agree with the figures of Harris [13] who, in guinea pigs weighing 250 gm., noticed a normal ratio of 4:10 in the epiphyseal line of the upper tibia.

Chondrophyte: As early as 1 week following testectomy a

marked proliferation and hypertrophy of the euhyaline cartilage cells is seen. Consequently, typical basophilic "incubator" or "brooding" capsules of cartilage are produced. Because of the increase in number and size of cartilage cells, the intercartilaginous stroma is diminished in amount. It may reveal a slight tendency to basophilia. Toward the peripheral part an accelerated conversion of precartilaginous cell elements into mature cartilage cells takes place. These changes become even more accentuated at later stages following castration (Fig. 2). A fusion of hypertrophied cartilage cells may occur. Occasionally, multinucleated giant cell-like structures which may become dissolved are found, or, here and there, a degeneration of individual cartilaginous cells may take place. When the nuclei disintegrate the finer cellular structure disappears and the cytoplasm becomes vacuolated and lumpy, and shows irregular outlines.

As in the epiphyseal line, so also in the chondrophyte, the hyperplasia and degeneration of the cartilage cells are evidently more pronounced in *males* than in *females*. In the latter, extensive basophilic incubator capsules and degeneration have not been observed.

Joints: In *males* the cartilage cells of the surface of the joint are increased in number (Fig. 3 a). In quite a few areas 4 or more cells may be packed into one capsule. The acidophilic intercartilaginous ground substance is diminished in amount. The hyperplastic process affects primarily the cartilage cells of the transitional and pressure zone. This proliferation becomes marked about 2 to 3 weeks following castration. On the other hand, hypertrophic changes are only slight and, if they occur at all, usually occur at the earliest 3 to 4 weeks following castration. In addition, capillary loops of the bone marrow may protrude through the zone of ossification into the pressure zone and corrode the cartilage cells of the latter. These changes indicate the beginning of arthropathic lesions of a definite but not acromegalic type.

In *females*, 1 week following ovariectomy a hyperplasia of the cartilage cells within the various zones takes place accompanied or followed by a marked hypertrophy of the cells. At later stages (Fig. 3 b) a liquefaction of the hypertrophied cartilage cells may occur and give rise to lesions resembling in character the acromegalic arthropathia which we have described previously [9] as tak-

ing place after the administration of acid extract of anterior pituitary, although under the latter condition they are less marked than after castration.

Thus, the cartilage of the female joint shows in the first place a greater responsiveness than that of the male, and secondly a greater tendency to hypertrophy. This behavior agrees with that of the cartilage of the epiphyseal line in the two sexes.

Bone Marrow: The capillaries are congested. Strands of hypertrophic, incompletely ossified or non-ossified cartilage may be surrounded by osteoblasts laid down in ribbons along the outlines of these strands. They are accompanied by a more or less fibrous connective tissue. This condition is most pronounced 2 to 3 weeks subsequent to castration. The rather loose connective tissue may extend a greater or shorter distance into the metaphysis and toward the lymphoid marrow. Many mitoses of blood and connective tissue cells are seen everywhere in areas adjacent to the cartilaginous cell layers.

In *females* the marrow is likewise congested. The tendency to fibrosis is slight and is practically negligible. Strands of hypertrophied calcified cartilage were not seen.

Cortex: The periosteum and endosteum in both sexes are apparently intact. There is no evidence of a disturbance in the equilibrium between apposition and resorption of bone.

Ribs: In *males* the intercartilaginous stroma reveals retrogressive changes which increase with increasing intervals after castration. During the first 2 weeks swelling and degeneration predominate in the stroma, whereas during the 3rd week degeneration of the cartilage cells becomes noticeable, and this may reach a high degree during the 4th and 5th weeks (Fig. 4 a). The cells affected are found in the columnar cartilage of the chondro-osseous junction as well as in the resting cartilage of the cartilaginous part of the ribs.

In *females*, in accordance with what we observed in other cartilaginous tissues, the tendency toward degeneration is less marked. Hyperplasia of cells may occur but is less accentuated than in the males.

Vertebrae: The zone of endochondral ossification reveals conditions analogous to those seen within the epiphyseal line of the tibia. Degeneration as well as hyperplasia is more pronounced in

males, but hypertrophic changes are more frequently found in *females*.

IV. Gonadectomized and Injected Animals:

Epiphyseal Line: In *males*, as the number of injections increases the epiphyseal line of the tibia becomes progressively narrower than in castrated non-injected animals. The degenerative processes (Fig. 5) within the chondromucoid substance are evidently more accentuated and more extensive than in either castrated or injected control animals. A marked hyperplasia and hypertrophy of the cartilaginous cell elements becomes noticeable. However,

TABLE IV

Ratio of the Number of Hypertrophic Cartilage Cells to the Number of Proliferating Columnar Cartilage Cells

Experiment	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks
Normal males and females	4–5:11	4:10–11	4:12	4:10–11	..
Testectomized and injected animals	5:16	4–5:17	5:18–19	6:18–19	..
Testectomy followed by 7 injections during the last week	..	6:16	5:16–17	3:15	3–4:14
Ovariectomized and injected animals	5:13	4:11	5:11	5–6:10	..

the hypertrophy does not exceed the degree obtained by castration alone. At later stages the pericellular calcium deposits cause a dense incrustation of the hypertrophic cartilage cells. Moreover, lines of deposited calcium are seen within the matrix of the columnar cartilage layer. But, even after 28 injections a complete calcification of the epiphyseal line, as found after the administration of the extract alone, was not observed. Differential counts of the number of proliferating and hypertrophic calcifying cartilage cells gave results as shown in Table IV.

The changes tabulated in Table IV are seen after a period of at least more than 7 days, during which a combination of castration and injections of extract was effected.

Chondrophyte: In *males* the hyperplastic and hypertrophic

growth as seen after either castration or injections of the extract alone may be intensified. Consequently, a number of incubator capsules degenerate, taking a dark blue stain with hematoxylin without revealing any structural details (Fig. 4 b). In the corresponding *females* the hypertrophic and degenerative changes are less pronounced.

Joints: In comparison with either castrated non-injected animals, or with non-castrated injected animals, the cartilage reveals a higher degree of response to the combined action of castration and acid extract. In *females* a marked hypertrophy is associated with a distinct hyperplasia. The cells of the sliding zone are not only increased in number, but the cells of the transitional layer take on a perpendicular arrangement, their cytoplasm at the same time increasing in size. Some cells may degenerate and liquefy, thus leading to the formation of minute cavities. The cells of the pressure zone are likewise hypertrophic. In addition, loops of capillaries and marrow cavities penetrate through the zone of ossification and invade the pressure and transitional zone, causing an increased resorption of the cartilage and corrosion of the surrounding bony lamellae. Thus, arthropathic lesions result.

In *males*, although hyperplastic growth occurs in the transitional layer, hypertrophic changes take place only after long treatment with the extract and never reach the same extent as in females. Consequently, arthropathic lesions are less frequently seen in males.

Bone Marrow: As in the case of castrated *males*, the bone marrow contains strands of hypertrophied calcified cartilage cells which are longitudinally arranged and suggest the shape of trabeculae, although ossification has not as yet or has only incompletely taken place. These strands may be surrounded by osteoblasts. The greater the number of injections given, the thicker, more cellular and massive are these strands. They are accompanied by a mass of fibrillar connective tissue which in some cases extends throughout the width of the subepiphyseal area of the bone marrow and is sharply demarcated from the normal metaphyseal bone marrow (Figs. 6 and 7). As a rule the maximum of fibrosis is reached after the 2nd week. At later stages the strands of cartilage and the fibrosis recede gradually.

As compared with the males, in *female* ovariectomized and in-

jected guinea pigs the cell proliferation is definitely decreased, the tendency to calcification and ossification is less accentuated, and the equilibrium between growth of cartilage and replacement by bone is well maintained. The border line between the zone of calcification and ossification is regular and only a few strands of calcified cartilage within the bone marrow were seen in 1 case.

Cortex: On the whole in both sexes the appositional and resorptive processes are stimulated but generally well balanced.

Ribs: The degenerative changes within the chondromucoid matrix as well as in the cartilage cells may be intensified as compared with those seen in either merely castrated or injected animals. A great number of cartilage cells are degenerated, revealing a disintegrated cytoplasm stained a deep blue with hematoxylin. As in the epiphysis of the tibia, wide areas are degenerated and entire cartilage cell rows may be affected. Furthermore, a distinct hyperplasia and hypertrophy of the resting and columnar cartilage cells are seen so that the stroma is diminished in amount. The marrow in the subepiphyseal layer is fibrous in nature. In 2 cases, in which following testectomy acid extract was injected over a period of 4 weeks, a broad ribbon of conglomerated hypertrophic cartilage cells was detected within the bony part of the rib, far away from the zone of ossification. At present it is difficult to understand why ossification failed to take place in this area. A fracture could be excluded in this instance.

Also, in the ribs the retrogressive lesions are more frequent in *males* than in the corresponding *females*.

Vertebrae: The behavior of the endochondral ossification in the vertebrae corresponds to that noted within the epiphyseal line of the tibia. Moreover, under the influence of the extract, in castrated and injected *male* guinea pigs, the occurrence of osseous plugs is a rather regular phenomenon (Fig. 8). In *females* they likewise occur but are much less frequent.

In the instances in which 7 injections were given after certain periods of time had elapsed since castration, the cartilaginous cells revealed an increased tendency to hypertrophic and degenerative changes in the chondrophyte and the joint. This increase was more notable the longer the interval between castration and the beginning of the injections. The columnar cartilage of the epiphyseal line showed a decreasing tendency to proliferation, whereas

hypertrophy was at first increased and then dropped below the normal level.

Comment

Our investigations have shown that both injection of acid extracts of anterior pituitary of cattle and gonadectomy cause proliferative as well as retrogressive changes in the euhyaline cartilage of the immature guinea pig. These effects are not limited to the epiphyseal line of the long bones but extend to various parts of the skeleton. As stated above, in both sexes characteristic changes may take place in the epiphyseal line of long bones, joints, bone marrow, bony cortex, ribs and vertebrae. They consist of hyperplasia and hypertrophy of the cells as well as degeneration of cells and intercartilaginous matrix, increased calcification of the hypertrophic cartilage, and a tendency of the bone marrow to fibrosis.

The histological appearances vary and depend upon the degree in which the various tissues participate in these processes. This participation may be determined by hormonal stimulation, by factors inherent in the cells and tissues, or by both of these factors. Thus, under certain conditions one of the following changes may predominate: (1) proliferation of cartilage cells; (2) the process of calcification of hypertrophic cartilage; and (3) ossification of calcified cartilage cells.

(1) *Cell Proliferation:* This occurs in gonadectomy as well as after injection of small amounts of extracts and after implantation of anterior pituitary of cattle. This suggests that the growth-promoting effect of gonadectomy on the cartilage may be secondary to an increased production or liberation of the growth hormone of the anterior lobe of the pituitary gland. This might well be the case because it has been shown by Friedman and Loeb [14] that the stimulating effect of gonadectomy on the pituitary gland is a non-specific one, inasmuch as accompanying the increase in gonadotropic activity the thyrotropic action of the pituitary gland is also enhanced. The increased formation of cartilage cells similarly may be explained as being due to an increased growth-promoting activity of the anterior pituitary gland. Although following gonadectomy proliferation of the cartilage is activated in both sexes, it is the male in which the growth stimulation is more intense. This

would mean that the testicle exerts a stronger inhibition on the pituitary gland than does the ovary.

In this connection it is noteworthy that in gonadectomized male guinea pigs the proliferative activity of the thyroid gland is greater than in the corresponding females,[15, 16] an effect which may perhaps be due to an increased stimulation of this gland by the male pituitary gland after removal of the inhibiting action of the testicle. However, this interpretation is at present merely of a tentative nature.

(2) *Calcification:* In males the process of calcification of the hypertrophic cartilage is obviously more interfered with than in females, as evidenced by the appearance in the former of hypertrophied, heavily calcified cartilaginous strands. The question now arises whether calcification of the hypertrophic cartilage is also regulated by hormonal influences or whether it is due to nutritional disturbances, or to conditions inherent within the cells. Harris [13] contends that because of nutritional conditions a given mass of cartilage is not able to grow indefinitely but is subject to retrogressive changes as soon as a maximum size has been reached. In our experiments this is apparently true of the chondrophyte and the ribs, where a more or less extensive degeneration of matrix and cells occurs. However, conditions within the epiphyseal line seem to be different, and factors other than the maturation or senescence of the cartilage must be involved in the process of calcification and subsequent ossification. Under normal conditions a single cell row of the epiphyseal line consists of about 10 to 11 columnar, and 4 hypertrophic cartilage cells. Since calcification of the cartilage takes place at this level it should be expected that these numbers represent the maximum beyond which the cartilage cells cannot multiply without undergoing degeneration. This is not the case, however, in gonadectomized animals. In the gonadectomized female 6 hypertrophic cartilage cells are frequently found, while the number of cells in the proliferating columns averages 19. Furthermore, in such animals the epiphyseal line is much wider than in normal guinea pigs and its total volume is also considerably increased. Notwithstanding the increased volume, the senescence of the cartilage, as determined by its tendency to calcification, is not only not increased but on the contrary rather retarded as compared with the normal. This gain in the power of

resistance of the cartilage cells under these conditions may be due to changes in the action of certain hormones.

As to the process of calcification of the hypertrophic cartilage, it need not necessarily be associated with disturbances of the calcium level in the serum, but it seems to depend on changes in the tissues themselves which are induced by the hormones. But it is very unlikely that there exists a direct relation between calcification of the cartilage and the action of the pituitary gland in view of the fact that in ovariectomized animals the calcification of the cartilage is diminished in comparison with the normal, whereas in male castrated animals it is increased. If the pituitary should be involved one would expect the castrated female to show a degree of calcification intermediate between the normal and the male castrate. We must consider, however, the possibility that the thyroid or the sex glands are involved in this process. We have begun experiments to test this suggestion.

(3) *Ossification:* The mechanism of ossification is subject to factors similar to those active in the process of calcification of the cartilage; hormonal influences as well as local environmental conditions must be considered. Ossification depends on the action of certain constituents of the bone marrow on the calcified cartilage. However, in the course of our experiments it was possible under certain conditions to observe a partial dissociation between the process of calcification and ossification of the cartilage. A partial dissociation occurred when cartilaginous strands underwent hypertrophy and calcification but ossified only very incompletely. Such an insufficiency of ossification may result either from an overproduction of calcified cartilage or from a relative or absolute inability of the bone marrow to penetrate into the calcified cartilage and cause a breakdown of the cartilage cells. This condition is usually combined with a tendency to fibrosis of the bone marrow.

We have not as yet discussed the significance of *degeneration* within the epiphyseal line of male castrates. It might be tempting to correlate it likewise with the overproduction of cartilage cells. There are, however, certain considerations which render such a correlation improbable. In the first place we have observed similar degenerations in female guinea pigs, which had received 2 to 4 implants of one-quarter of a gland of anterior pituitary of cattle, and in which the number of the columnar cartilage cells was not

as high as in the case of castration of males at later dates. Moreover, if nutritional disturbances had been the cause of these degenerative changes, one would have expected the degenerated areas to be located in places farthest remote from the nutritional vessels, namely in the zone of calcifying cartilage and extending horizontally through the epiphyseal line (lines of arrested growth, Harris [13]). On the contrary, we find the areas of degeneration extending in a vertical direction all through the epiphyseal line parallel to the columns of cartilage. We may then assume that these degenerations, similar to those following implants of anterior pituitary, are due to a direct or indirect effect of the pituitary gland. Perhaps this condition can be tentatively explained in the same way as the calcification in males, namely as being due to the direct or indirect effect of the increased action of the anterior pituitary gland.

Summary

In immature guinea pigs gonadectomy causes an increased proliferation of the euhyaline cartilage in various cartilaginous tissues. In males hyperplastic growth is more accentuated, whereas in females a more pronounced tendency to hypertrophy is present. The latter is especially evident within the joint, where arthropathic lesions are initiated more readily than in males.

In males degeneration occurs more frequently and to a greater extent than in females. Calcification and ossification of the cartilage appear balanced in females, but in males marked disturbances are observed in the zone of ossification. Associated with these disturbances is a tendency to fibrosis of the bone marrow.

The cause of the increased proliferation is seen in a non-specific stimulation of the anterior pituitary subsequent to gonadectomy. Differences in the behavior of the sexes are tentatively explained as due to differences in the inhibiting action of the gonads on the pituitary gland in males and females, and also to differences in the action of gonadectomy on the thyroid gland in the two sexes.

A combination of the action of acid extract of anterior pituitary of cattle and of castration leads to a summation effect which is determined by different degrees of stimulation exerted in the various tissues.

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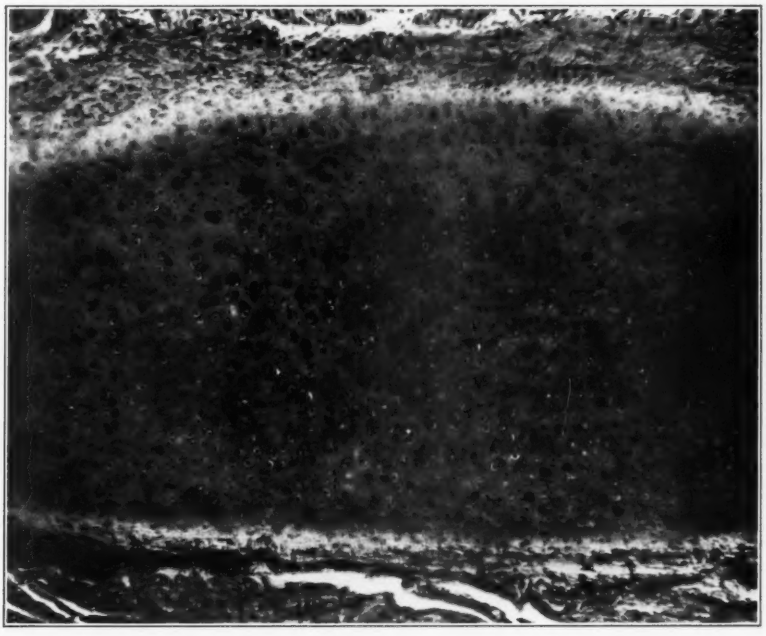
DESCRIPTION OF PLATES

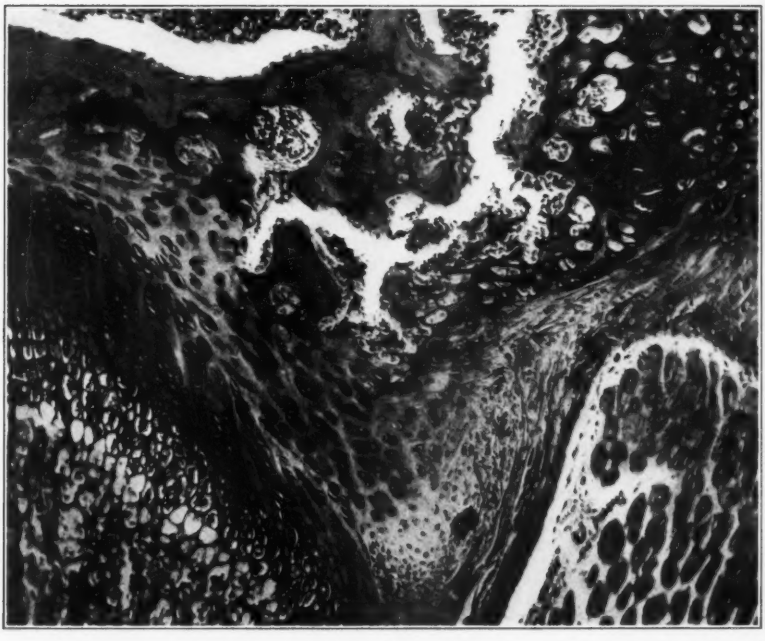
PLATE 12

FIG. 1. Cartilaginous area of a rib from a male guinea pig which received 8 injections of acid extract of anterior pituitary of cattle. Numerous, deeply staining degenerated cells which do not show any structural details are seen.

FIG. 2. Male guinea pig 3 weeks after testectomy showing hyperplasia and hypertrophy of the chondrophyte with incubator capsules. Hyperplasia and hypertrophy are present in the epiphyseal line.



1



2

Plate 13

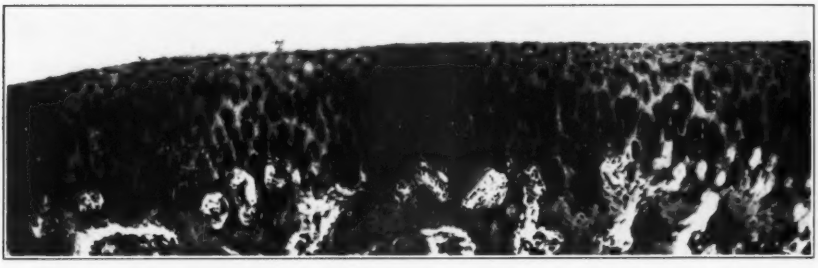
Fig. 3 a. Cartilage of the knee joint from a male guinea pig 4 weeks after testectomy. Marked hyperplasia but less hypertrophy is present than in the corresponding female (b).

Fig. 3 b. Knee joint of a female guinea pig 4 weeks after ovariectomy. Marked hyperplasia and hypertrophy of the cartilage cells of the various zones is seen. Corrosion of the basal pressure zone is produced by protruding capillary loops of the bone marrow.

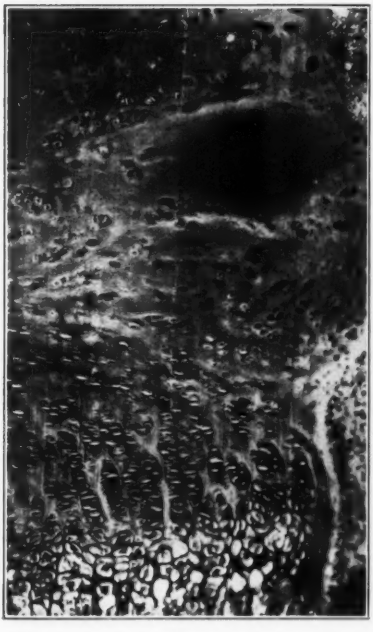
Fig. 4 a. Male guinea pig 5 weeks after testectomy. Degeneration of the cartilaginous matrix is present with destruction of cartilage cells near the chondro-osseous junction in the rib.

Fig. 4 b. Male guinea pig after castration and 12 injections of acid extract. Degenerated brooding capsules are present in the chondrophyte.

3 a



3 b



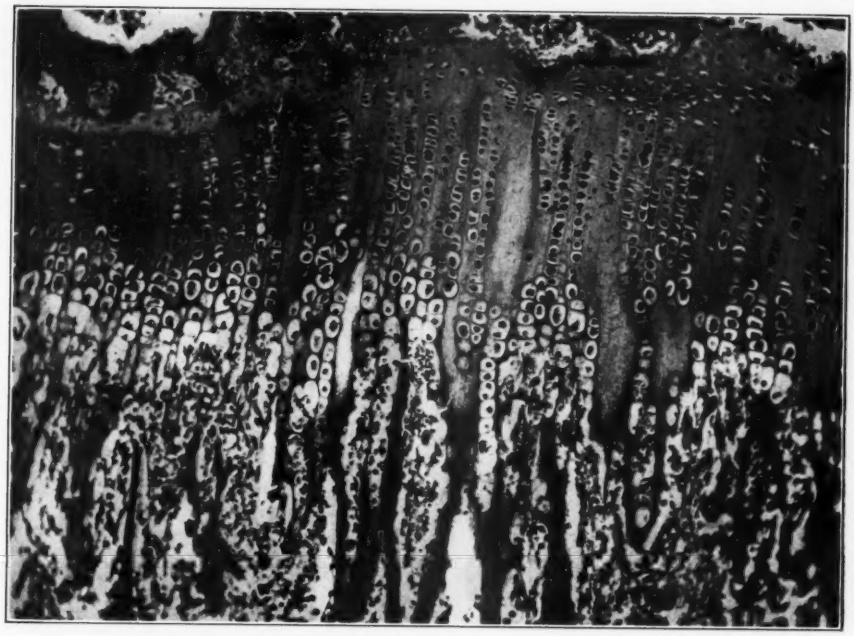
4 a

4 b

PLATE 14

FIG. 5. Male guinea pig after testectomy followed by 12 injections of acid extract of anterior pituitary of cattle. Degeneration of whole cell rows and proliferation of the cartilage cells of the epiphyseal line is seen.

FIG. 6. Male guinea pig after testectomy and 12 injections of acid extract of anterior pituitary of cattle. Calcified and incompletely ossified strands of hypertrophic cartilage are seen protruding into the bone marrow of the tibia. Note the lack of lymphoid marrow between the strands. The epiphyseal line is narrow.



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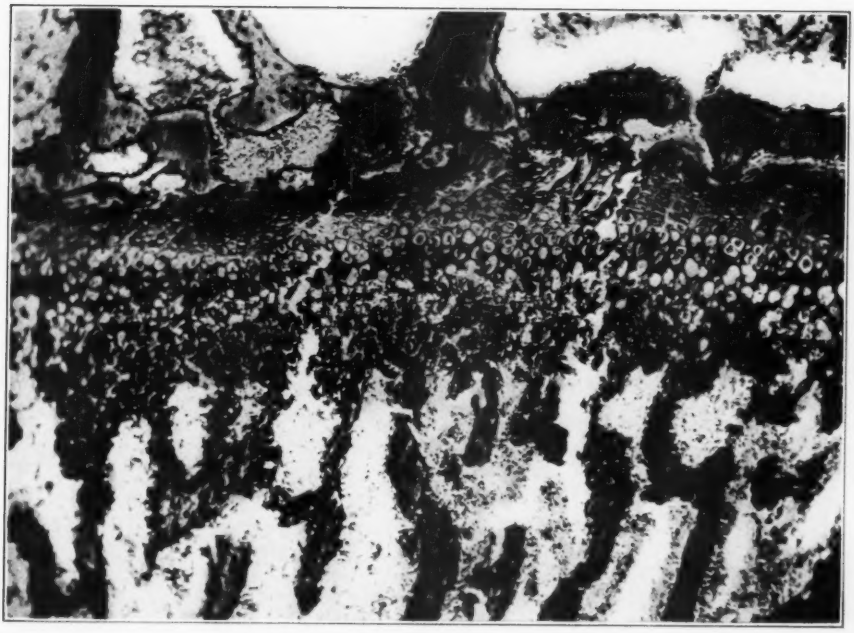

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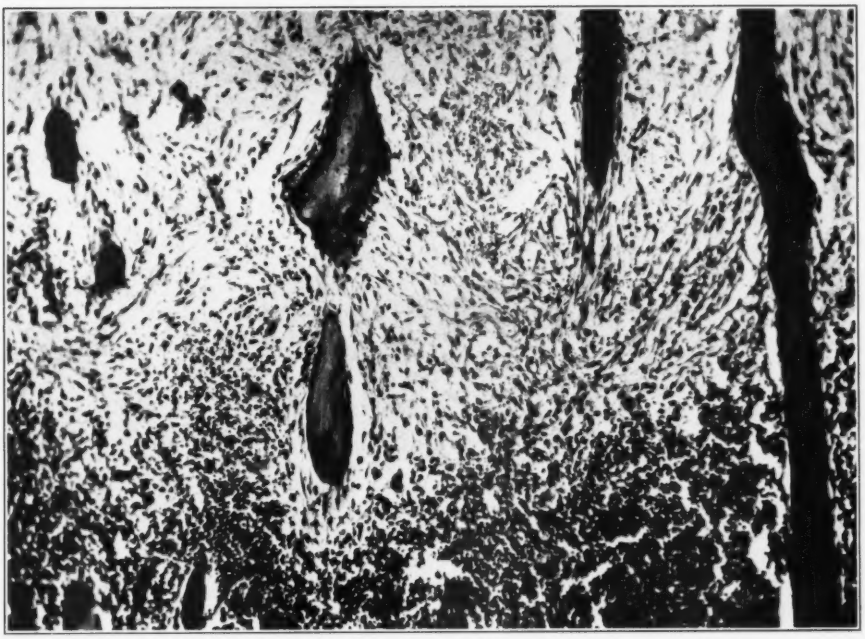
PLATE 15

FIG. 7. From the same guinea pig as shown in Fig. 6. Note the fibrosis of the bone marrow of the tibia. The lower part of the picture shows preserved lymphoid marrow.

FIG. 8. Male guinea pig after castration and 20 injections of acid extract of anterior pituitary of cattle. Section of vertebra showing hypertrophic and hyperplastic growth within the zone of ossification. Note the osseous plug with transverse cracks and two cavities in the center of the picture.



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8

OBSERVATIONS ON THE ETIOLOGY OF ULCERATIVE COLITIS *

III. The Distribution of Lesions and its Possible Significance

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In 1853 Virchow [1] described the distribution of the lesions in colitis as follows †: "In particular it is the projections of the mucous membrane, caused by the anatomical distribution of the muscle tissue, which are affected by preference. Hence the especial susceptibility of the mucosa along the insertion of the three longitudinal muscle bands, and of the transverse folds of membrane. In that the diphtheritic process establishes itself first on the projecting and later on the intermediate mucosa, there arise those regular geographical figures which indicate the intensity and mode of spread of the lesion."

Virchow attributed this peculiar distribution of the ulcerations in colitis to the closer contact of the fecal current with the mucous membrane in places where the lesions were most pronounced. Although this interpretation is scarcely tenable, his description of the ulcerations according to a muscular pattern is most accurate. Pathologists in general have overlooked this statement by Virchow and no one has ventured to explain the arrangement of the lesions in ulcerative colitis. It is the purpose of this paper to reconsider the location of the ulcerations in this disease and to advance a possible explanation for their occurrence.

During a period of 18 months 6 cases of ulcerative colitis came to autopsy at the Mallory Institute of the Boston City Hospital. Two of these patients had been ill with their first attack of ulcerative colitis only 2 months before death. Four had had previous attacks and died during an acute exacerbation of the disease. Cultures of stools and agglutination reactions for dysentery organisms were negative on five patients. On one, cultures of

* Received for publication June 25, 1938.

† Translated by Flexner and Sweet.[2]

stools for pathogenic bacteria were repeatedly negative but *B. enteritidis* was recovered from the blood stream at autopsy.

In this group of cases the disease varied in its extent. One individual showed involvement of the rectum and sigmoid only; in three the lesions extended to the splenic flexure; in one to the midportion of the transverse colon; and one showed involvement of the entire colon without extension beyond the ileocecal valve. In all instances the most severe lesions were in the rectum, the walls of which were greatly thickened and indurated. The mucosa of the rectum was irregularly ulcerated. At the junction of the rectum and sigmoid the ulcerations developed into three rows running in the longitudinal direction of the intestine. Between these rows of ulcers the mucosa was edematous and indurated, and showed small erosions. In places there were ulcerations extending between the longitudinal rows of ulcers, and where this occurred they seemed to follow a linear pattern in the circular direction of the intestine. The bases of the ulcers were made up of the circular muscle fibers, and in most instances there was an undermining of the epithelial edges.

When the fat and mesenteric attachments were dissected away from the muscular layers of the intestine where this linear arrangement of ulcers was noted it was found that the lesions lay directly over the tenial bands. This correlation was found in all 6 cases. A possible explanation for the failure to note this distribution of ulcers previously is the manner in which the colon is usually opened at postmortem examination. Unless the wall between two tenial bands is opened deliberately, the teniae will frequently be transected in an irregular manner, thus obscuring the linear pattern of the ulcers.

Figures 1 and 2 show photographs of specimens taken from 2 cases of chronic, non-specific ulcerative colitis. The linear character of the ulcerative processes in the longitudinal direction of the intestine is clearly shown. Beneath each of these rows of ulcerations lies a tenial band. Figure 3 shows a low power microphotograph of another specimen in which a cross section of the colon reveals the manner in which the ulcerative process overlies the tenial band.

As mentioned previously, the intestinal tract was unevenly involved by the disease and lesions were found in all stages of

development in the same individual. The earliest changes noted in gross consisted essentially of hyperemia, edema and petechial submucous hemorrhages. In certain areas where the lesions had advanced to a later stage, superficial erosions occurred which were covered with fibrin. Other lesions were found which were interpreted as transitional stages between the earliest erosions and the frank ulcerations. A peculiar feature of the sections taken from these early lesions was the complete or almost complete absence of mucus in the epithelial cells. This finding was noted in all sections taken from the acutely involved areas and contrasted sharply with the appearance of the mucous membrane in a normal colon. Figure 4 shows a section of normal human colon with great numbers of goblet cells. Figure 5 shows for comparison a section from a patient who died from acute ulcerative colitis. The absence of mucus-containing goblet cells is quite striking.

Discussion

The peculiar pattern of the lesions in ulcerative colitis cannot be a mere accident, and an interpretation of their location may throw light on the etiology of this disease. The rectum possesses the most powerful muscles in the colon.[3] It differs from the rest of the colon in that it has no teniae and the longitudinal fibers are evenly distributed about its circumference. Above the level of the rectum the tenial bands are the most powerful part of the colonic musculature.[4, 5] The arrangement of the severest lesions in ulcerative colitis is such that they lie directly over the most powerful muscles, and there may well be a direct relation between the muscles and the lesions.

In this connection it is interesting to note that the early stage of ulcerative colitis can be simulated experimentally in animals by hypermotility and spasm of the colonic muscles. Elsewhere [6] one of us has reported experiments on explants of the colon in dogs. These explants were subjected to severe muscular spasm by the use of prostigmin intravenously and acetylcholine salt locally. The changes produced by these methods consisted first of diffuse hyperemia and edema. Within a few minutes petechial hemorrhages were noted beneath the surface epithelium, and later superficial erosions with hemorrhage oozing from the surface and marked induration of the explant appeared.

The microscopic appearance of biopsied material taken from the explants after muscular hyperactivity was found to be essentially that of a diffuse inflammatory reaction in the mucosa. In addition it was noted that whenever the explant underwent severe muscular spasm all of the evident mucus in the goblet cells had disappeared. From these experiments it seems reasonable to conclude that if the colon is stimulated to intense muscular activity, it is capable of producing severe damage to the mucosa, similar to the pathological changes noted in early ulcerative colitis.

Spasm and hypermotility of the intestinal muscles are a prominent clinical feature of ulcerative colitis. They are manifested by diarrhea, tenesmus and urgency, by the pipe-stem colon on X-ray examination, and by the contracted segment of lower intestine on sigmoidoscopy, which distends only with difficulty or not at all under positive pressure. It seems highly probable in view of these considerations that ulcerative colitis is primarily a disease caused by intense muscular spasm and hypermotility, and therefore the distribution of lesions follows a muscular pattern. Whatever infectious element is present may well be due to secondary involvement of the damaged areas caused by muscular overactivity.

Summary and Conclusions

In 6 cases of ulcerative colitis it was demonstrated at autopsy that the severest lesions were in the rectum and overlying the tenial bands. This distribution of lesions over the most powerful muscles of the colon may well be due to the fact that hypermotility and spasm of the colonic muscles cause the disease. Supportive evidence for this interpretation is the fact that early lesions of ulcerative colitis have been produced in the colons of dogs by hypermotility and spasm of the muscles alone.

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DESCRIPTION OF PLATES

PLATE 16

FIG. 1. Specimen of colon from a patient (A 38–328) who died from acute non-specific ulcerative colitis. The ulcers have been painted with India ink to make them apparent and show more clearly the three rows of lesions. These rows of ulcers lie directly over the teniae.

FIG. 2. Section of colon from a patient (A 37–371) dying from non-specific ulcerative colitis. There are two rows of ulcerations in the longitudinal direction of the intestine, and they lie directly over the teniae. The third tenial band was cut away when sections were taken. Between the two teniae shown there are no ulcerations, only edema and hyperemia of the mucous membrane.

FIG. 3. This section is from the region of a tenial band in a patient (A 37–108) who succumbed to chronic non-specific ulcerative colitis. The ulcerative process is shown directly over a tenia.

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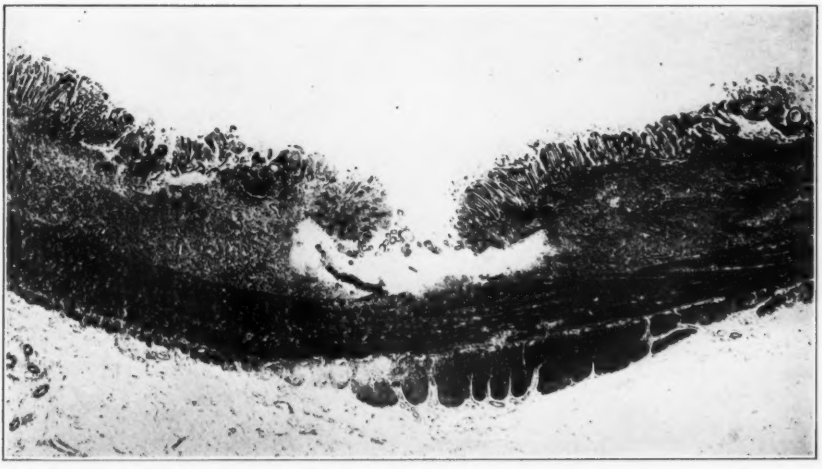

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PLATE 17

FIG. 4. Mucous membrane of normal human colon. There is only a narrow space between the glands and numerous goblet cells are present. × 200.

FIG. 5. Portion of mucous membrane from a case (A 38–10) of acute ulcerative colitis. Note the absence of goblet cells. This is similar to the changes produced by spasm alone in an explant of the dog's colon. × 200.

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5

Lium and Porter Etiology of Ulcerative Colitis. III

POLYARTERITIS NODOSA *

Report of Case

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A patient who had been under observation at the Massachusetts General Hospital for asthma for 8½ years finally died of polyarteritis nodosa. The autopsy findings are considered sufficiently interesting and unusual to warrant reporting the case.

Report of Case

Clinical History: A 30 year old white stenographer entered the hospital for the fifth time with the complaints of fever, pain in the chest, abdomen and joints, and recurring asthmatic attacks. For the 8½ years previous to her final entry she had suffered from recurring attacks of typical asthma. Her four previous entries had all been for this reason, and on three occasions she had been in status asthmaticus. She had been treated by various means, including psychotherapy, without lasting results. There was nothing in the physical examination or laboratory findings inconsistent with the diagnosis of asthma. Her blood had shown an eosinophilia varying between 5 and 13 per cent.

One month before final entry she experienced some nausea and, for the first time, developed pain in the right lower chest and upper quadrant which radiated around to the back. During the next week the pain centered in the left lower chest posteriorly, and she began to have intermittent fever. She also had some stiffness in the shoulders. Three days before entry the elbows and wrists became red, swollen, stiff and painful. The following day painful red spots appeared beneath the nails of the fingers and toes. The evening before entry an asthmatic attack recurred in severe form and the elbows and wrists became very painful. She also had some nausea and vomiting, and had lost about 20 pounds in weight during the previous 4 months.

Physical Examination: This revealed a poorly developed and nourished woman in considerable respiratory distress. The lips and mucous membranes were pale and the nail beds were cyanotic. On the finger-tips and toes were many ham-colored lesions grouped together but not elevated. There were a few similar discrete lesions on the feet, and there were many petechiae beneath the fingernails and toenails and on the abdomen. A few, moist and sibilant râles were present in the lungs. No masses could be palpated in the abdomen, but there was diffuse tenderness and spasm in the epigastrium and right upper quadrant. The wrists were slightly stiff, tender, and painful with movement, and there was slight swelling on the dorsal aspects. Motion of the elbows was very painful so that they could not be fully extended. The blood

* Received for publication August 3, 1938.

pressure was 95/60, the pulse 120, the temperature 102° F., and the respirations 28.

Laboratory Examinations: The urine had a specific gravity of 1.022, contained 1+ albumin, and showed occasional red cells, white cells and granular and hyaline casts. The blood showed a red cell count of 4,000,000 with 75 per cent hemoglobin and a white cell count of 43,000, with 60 per cent polymorphonuclears, 18 per cent lymphocytes, 2 per cent monocytes and 20 per cent eosinophils. The platelet count was slightly below normal. The guaiac test on the vomitus was negative and on the stool 1+ positive. A blood Hinton test was positive, and a Wassermann test negative. The non-protein nitrogen of the blood was 24 mg. per cent, the protein was 61 per cent, and the chlorides were equivalent to 103 cc. of N/10 sodium chloride. Several blood cultures were negative.

An electrocardiogram showed moderate to marked left axis deviation and was interpreted as consistent with coronary heart disease.

A roentgenogram of the chest showed marked increase in the size of the heart, particularly on the right side in the region of the pulmonary conus. The hilar shadows were slightly increased in width and there was marked increase in the lung markings, particularly in the hilar regions and lower lung fields. Diffuse haziness was present above the diaphragm suggestive of a pneumonic process. Three weeks later another roentgenogram showed disappearance of the apparent pneumonic process with persistence of the other findings. A postmortem roentgenogram showed the hilar shadows to be smaller and the lung markings to be essentially unchanged. In the peripheral lung fields there appeared to be an early miliary process.

Course of Illness: During her stay in the hospital the white count varied between 25,000 and 45,000, the eosinophils rising from the initial 20 per cent to 40 and 50 per cent. The pulse remained elevated although the temperature fell to normal on the 11th day. The abdominal pain continued and spread into both flanks. Both the liver and spleen became easily palpable. She had transient blindness of the left eye lasting for 2 days. On the 36th hospital day she developed severe, migrating neuritic pains in the extremities which caused a great deal of distress. The course of illness was marked by progressive weakness and emaciation, and death occurred on the 45th hospital day. A clinical diagnosis of polyarteritis nodosa was made on the basis of the history of severe asthma, irregularly distributed pain in the joints and muscles, pain in the abdomen and chest, skin lesions, evidence of renal and cardiac damage, emaciation, and marked eosinophilia.

Postmortem Examination

At autopsy no gross arterial lesions were discovered. The terminal ileum and colon showed numerous superficial areas of ulceration which were for the most part oval in shape, measuring up to 1.5 cm. in length, with long axes running transversely.

The heart weighed 325 gm. The right ventricle was somewhat dilated, and embedded beneath the endocardium of the left ventricle was a firm, dull gray nodule measuring 2 mm. in diameter.

Both lungs were similar in appearance. Scattered throughout the parenchyma, most marked in the right lung and least marked in the left upper lobe, were numerous grayish white nodules measuring 0.2 to 1 mm. in diameter, some of which were confluent. In the inferior edge of the left lower lobe was a triangular area of obvious infarction measuring 1.5 by 1 by 0.5 cm. No emboli were found.

In the left lobe of the liver and adjacent portion of the right lobe were large reddish areas of infarction.

The spleen weighed 825 gm. It was very soft and friable and the normal architecture was completely obliterated by almost confluent round areas of soft white tissue measuring up to 3 mm. in diameter with intervening areas of dark red hemorrhagic tissue. The splenic vein and artery were negative.

The adrenals and pancreas were grossly negative, but the kidneys showed a number of small areas of infarction measuring up to 3 mm. in diameter.

Several peripheral nerves and specimens of muscle appeared negative in gross.

The brain showed an area measuring 1.5 cm. in diameter of recently clotted blood in the posterior portion of the left lenticular nucleus.

Microscopic Examination

Microscopic lesions characteristic of polyarteritis nodosa were found in the heart, lungs, liver, pancreas, adrenals, kidneys, muscles, peripheral nerves and brain, but were not found in the ovary, aorta, lymph nodes, intestine or bone marrow. The size of the arteries affected varied considerably but none of them was larger than 1.5 mm. in diameter and few, if any, arterioles were involved. None of the affected vessels could be identified as veins. Although the colon showed extensive ulceration similar to that seen in tuberculosis, no microscopic arterial lesions could be demonstrated. Only ulceration and inflammation of an entirely nonspecific nature was seen. No gross or microscopic lesions were demonstrated in large vessels such as the coronary, mesenteric or renal arteries.

In this study four definite stages of the disease were found: (1) early acute stage; (2) advanced acute stage; (3) granulating stage; and (4) healed stage.

What appears to be the very earliest acute vascular lesion was limited to the vessels supplying the skeletal muscles. It consisted of apparent disintegration of the intima and innermost layers of the media of the arteries with infiltration of these areas by polymorphonuclear leukocytes, eosinophils and fibrin. In some blood vessels the internal elastic lamina remained intact, although it was usually partially destroyed. The middle and outer layers of the media were unaffected, and there was no periarterial thickening or inflammation. Many of the vessels were thrombosed.

Advanced acute lesions also occurred in the muscles, and in the pancreas, brain and nerve bundles. At this stage the intima and inner layers of the media were affected in all cases, and there were varying degrees of disintegration of the internal elastic lamina and muscular coats with loss of architecture and disappearance of many of the smooth muscle cells. The wall and often the adventitia were infiltrated by eosinophils and polymorphonuclear leukocytes. The mural disintegration did not always involve the entire circumference of the vessel. Frequently one-third or more of the circumference appeared perfectly normal with the muscle fibers and elastica preserved in their orderly arrangement. Often apparent aneurysmal dilatation of the affected segments of the vessels was seen, but there was no interstitial hemorrhage, probably because almost all these vessels were thrombosed. The lesions were characteristically segmental in their distribution. Longitudinal sections of the arteries showed involvement of as much as a millimeter of the wall without any damage to the adjoining segments on either side of the lesion.

Granulating lesions were seen in the lungs, adrenals and kidneys. There was ingrowth of fibroblasts into the affected portions of the wall and adventitia with decrease in the numbers of eosinophils and polymorphonuclear leukocytes, and increase in lymphocytes, plasma cells and large mononuclear cells. In this stage the adventitia was almost invariably involved in the process. In the more advanced granulating lesions the necrotic tissue of the acute stage was entirely replaced by cellular fibrous tissue which extended into the adventitia. The areas of the wall unaffected in the acute stage remained normal in appearance. There was also marked thickening of the intima by moderately cellular fibrous tissue, often with complete occlusion. It is difficult to say whether

the appearance was due to organization of a preexisting thrombus with or without recanalization or to intimal proliferation in a vessel that was not thrombosed. In at least a few of the more acute lesions the occluding acute thrombi showed very definite beginning organization, indicating that this process was responsible for some of the luminal occlusion seen in the later stages. An artery in the adrenal showed infiltration of the entire media with densely packed, blue staining, fibrin-like material. The rest of the lesion showed an advanced stage of granulation.

Healed lesions were seen in the liver and heart. In the liver the granulation tissue was replaced by dense, relatively acellular fibrous tissue containing a few pigment-bearing mononuclear cells and occasional foreign body type giant cells. In some of the lesions part of the media of the blood vessels could be identified with the underlying elastica intact. The lumen was almost invariably occluded and recanalized. In the heart all the lesions were healed, and only a few of the smallest arteries were affected. These, however, showed no definite involvement of the muscular layer. Marked fibrous thickening of the intima with partial or complete occlusion, or occlusion followed by recanalization was present. In some, the internal elastic lamina was partially destroyed but in the majority it was intact.

The histological appearance of the spleen was most unusual. With the exception of a few very small follicles around persisting trabeculae, the normal architecture was almost completely obliterated. There was massive infiltration of the parenchyma by large numbers of monocytes and red cells with a few lymphocytes and moderate amounts of fibrin. In some areas the fibrin was arranged concentrically around collections of cells giving a follicle-like appearance. There were no areas of necrosis, no fibrosis, and no suggestion of tubercle formation. Sections stained for tubercle bacilli showed no acid-fast organisms, and material inoculated into guinea pigs failed to produce tuberculosis. Very extensive degeneration of the walls of many of the arterioles was present, but no infiltration by eosinophils or polymorphonuclear leukocytes and no periarteriolar thickening was seen. Many arterioles were almost unrecognizable. Some of them were thrombosed and only a few were normal in appearance. However, no definite specific lesions such as occurred in other organs could be identified. Some

of the small arteries showed infiltration of the adventitia by lymphocytes and plasma cells, and in many of the veins there were small subendothelial collections of mononuclear cells elevating the endothelium and encroaching upon the lumen.

In the lungs were widespread miliary foci of organizing pneumonia involving areas which would normally be occupied by one to ten alveoli, although some of them were considerably larger. These foci consisted of interlacing fibrous tissue with a few mononuclear cells and eosinophils, occasional small foreign body type giant cells, and small amounts of fibrin. No areas of caseation necrosis were present, and no acid-fast organisms could be identified in sections stained for tubercle bacilli. In many of the larger branches of the bronchial arteries measuring 0.5 to 1.5 mm. in diameter were specific lesions in the advanced stage of granulation, most of which were located near the hili. In some of the pulmonary arteries were lesions that appeared to be organized mural thrombi. They were suggestive of specific lesions, but without destruction of the arterial walls or infiltration by inflammatory cells they could not definitely be considered as such.

Varying stages of ischemic necrosis were present in the heart, liver, kidneys, adrenals and pancreas. However, the stages of infarction did not necessarily correspond with the apparent age of the corresponding arterial lesions. In the heart where the arterial lesions were healed were a few areas of acute necrosis of the muscle bundles, although there were present also numerous small areas of healed infarction. In the liver all the infarction was early; whereas the majority of the arterial lesions were healed. In the kidneys areas of early infarction and also areas where the tubules were degenerated without damage to the glomeruli or interstitial tissue were present. The adrenals showed several areas in the cortex where the cortical cells were completely replaced by macrophages filled with fat. A few small areas of early ischemic necrosis were seen in the pancreas.

Comment

In all the arterial lesions observed there was some degree of damage, with or without repair, of the intima and almost invariably of the inner layers of the media. Aside from the evidence already offered this would indicate that in this case the primary lesion

occurred in the intima and progressed outward through the wall to the adventitia.

Arkin [1] has classified the lesions in four stages. The first is an alterative degenerative stage. The elastica interna and innermost layers of the media become edematous and infiltrated by a thready fibrinous exudate, and part of the muscle layer of the entire wall undergoes a coagulation necrosis with beginning infiltration by leukocytes. This stage corresponds roughly with the early acute stage described in this case but differs from it in that the lesion is not confined to the innermost layers of the artery. Arkin's acute inflammatory, granulation and healed stages correspond to the three later stages described in this case.

In any inflammatory process progression from the acute to the healed stage occurs, and the lesion of polyarteritis nodosa is no exception to this. The effect of the toxic agent is limited by the inflammatory response, and greater or less damage is done depending on the various factors operative in each individual instance. The extent of the healed lesion depends directly on the amount of damage done in the acute stage.

An unusual feature of this case was the coincident existence of all stages of the arterial lesions. In many of the cases reported [2-8] with detailed descriptions of the histological findings, the lesions showed some variation in their stage of development, but in this case variation from the earliest acute to the healed stage was observed. However, with one exception this marked variation did not occur in the individual organs. Except in the liver, all the arterial lesions of each individual organ were roughly in the same stage. Only acute lesions were seen in the pancreas, brain, muscles and nerve bundles; only granulating lesions were seen in the lungs, adrenals and kidneys; and only healed lesions were seen in the heart. The majority of the lesions in the liver were healed, but a few were acute. This restriction of lesions to one stage in the individual organs cannot be readily explained. From a purely speculative point of view it may possibly have some bearing on the pathogenesis of the disease. Although the lesions are widely distributed throughout the body with the primary damage to the arteries, each individual organ as a whole may have a specific reactivity or resistance which determines its response to the toxic agent.

The lungs in our case showed an unusual X-ray appearance similar to findings in the case reported by von Conta [9] (quoted by Spiegel [10]), and in the case reported by Herrman [11] and Wiener.[4] Von Conta described shadows ranging in size from "a lentil to a cherry" located near the hilus with specific lesions of the pulmonary arteries at autopsy. Herrman and Wiener described a diffuse perivascular infiltration spreading out uniformly and equally from both hilar regions with specific lesions of the bronchial and pulmonary arteries. In this case the hilar shadows were slightly increased in width with marked increase in the lung markings, particularly in the hilar regions and lower lung fields. A postmortem film showed a generalized early miliary process. At autopsy there were numerous specific lesions of the bronchial arteries near the hili with miliary foci of organizing pneumonia throughout the parenchyma. The pulmonary arteries and peripheral branches of the bronchial arteries showed very little involvement. This appearance by roentgen examination of perivascular infiltration spreading out from the hilar regions may be helpful in establishing the diagnosis in a suspected case of polyarteritis nodosa. It cannot, of course, be considered diagnostic.

The gross appearance of the spleen was the most striking feature of the case at autopsy. It was enlarged to more than four times its normal size and showed lesions suggestive of very extensive tuberculosis of an unusual variety. Microscopically the entire organ was involved by a hemorrhagic, apparently inflammatory process, which had no resemblance to tuberculosis or any other specific disease.

The gross autopsy findings as a whole simulated tuberculosis more closely than any other condition. The miliary process in the lungs, the splenic lesions, the small infarcts in the kidneys, and the ulceration of the colon gave a combined picture which could well have been produced by widespread tuberculosis. The infarcts of the liver, the cerebral hemorrhage and the absence of any primary focus of tuberculous infection were, of course, incompatible with the diagnosis.

A note may be appended about nomenclature. Many of the older writers, including Lamb,[12] believed the lesion to be primarily periarterial. More recent evidence presented by numerous writers and the findings embodied in this report make it reasonably cer-

tain that the lesions are not primarily periarterial. In 1907 Dickson [13] suggested that the name polyarteritis nodosa be adopted. Periarteritis nodosa is, without much question, a misnomer and for that reason should be dropped. Polyarteritis nodosa would be a suitable substitute.

Summary

A case of polyarteritis nodosa is reported with clinical findings which made possible an accurate clinical diagnosis. The outstanding symptoms and signs were a history of severe asthma, irregularly distributed pain in the joints, muscles, abdomen and chest, skin lesions, evidence of renal and cardiac damage, emaciation and marked eosinophilia.

A detailed description of the gross and microscopic autopsy findings is given with comments on a number of unusual features of the case. These unusual features are: (1) Evidence that the primary lesion occurred in the intima and inner layers of the media and progressed outward through the wall to the adventitia; (2) occurrence of all stages of arteritis from the earliest acute to the healed, but restriction of the lesions in each individual organ to roughly one stage; (3) remarkable parenchymatous lesions of the lungs and spleen; and (4) resemblance of the gross autopsy findings to widespread tuberculosis.

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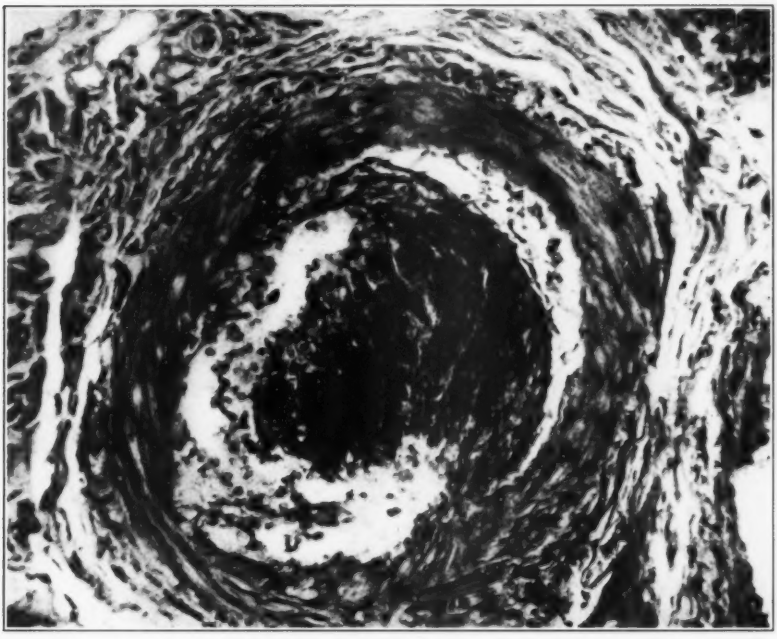
DESCRIPTION OF PLATES

PLATE 18

FIG. 1. An early acute lesion in a small artery of the biceps muscle. The intima and inner layers of the media are disintegrated and the lumen is occupied by a recent thrombus. There is no degeneration or cellular infiltration of the outer layers of the media, and no periarterial inflammation. Phloxine-methylene blue stain.

FIG. 2. A somewhat more advanced acute lesion of an artery 1 mm. in diameter in a nerve bundle. In the upper part the destruction of the wall is almost complete and there is beginning periarterial inflammation. Phloxine-methylene blue stain.



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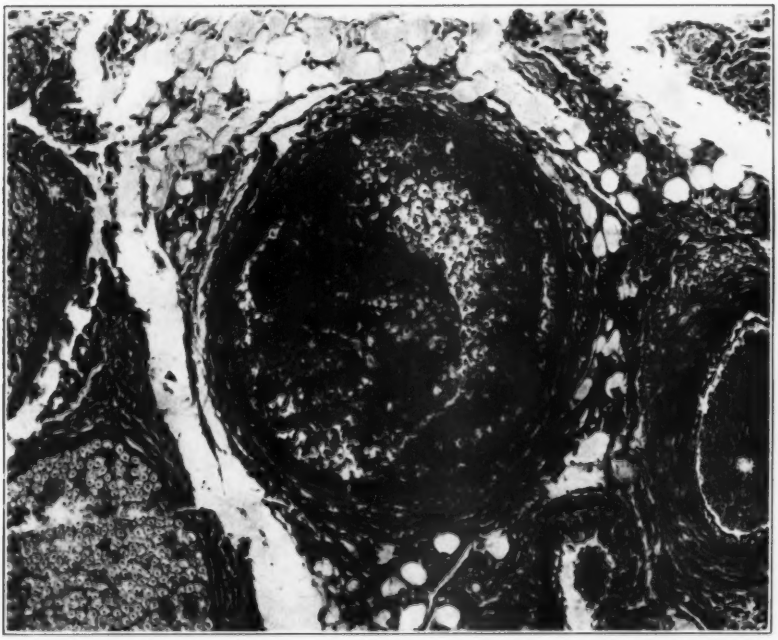

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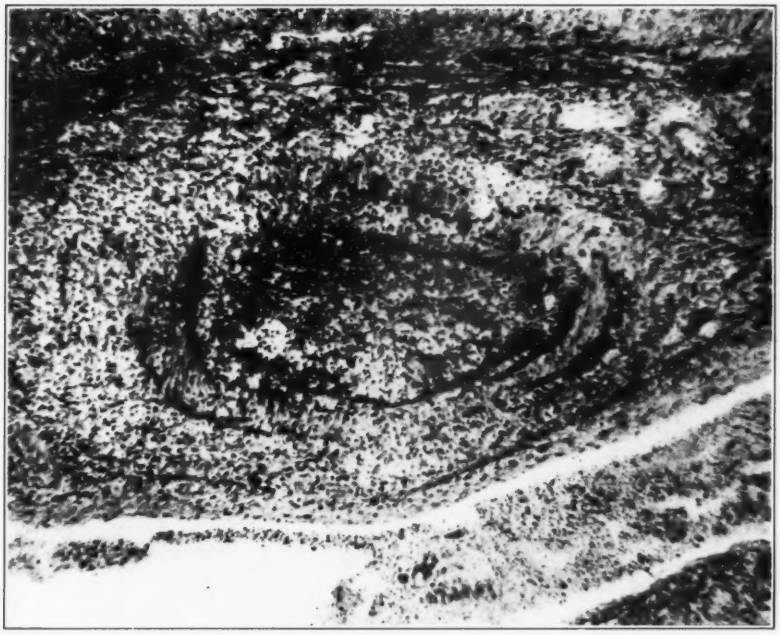
PLATE 19

FIG. 3. A granulating lesion of an artery 1 mm. in diameter in the kidney. There is almost complete destruction of the media except for a small area on the right where a segment of persistent media can be made out between the elastic lamellae. Verhoeff's elastic tissue stain.

FIG. 4. An advanced granulating lesion of a bronchial artery 1 mm. in diameter. Most of the inflammatory cells have disappeared and many small vessels have developed, partly in the granulation tissue which has replaced the media, and partly as recanalizing vessels in the thrombus. Verhoeff's elastic tissue stain.



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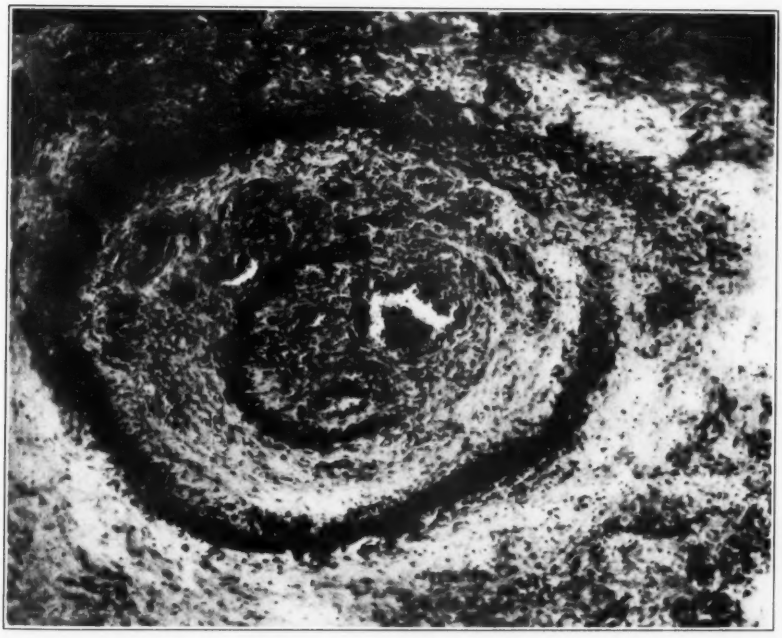

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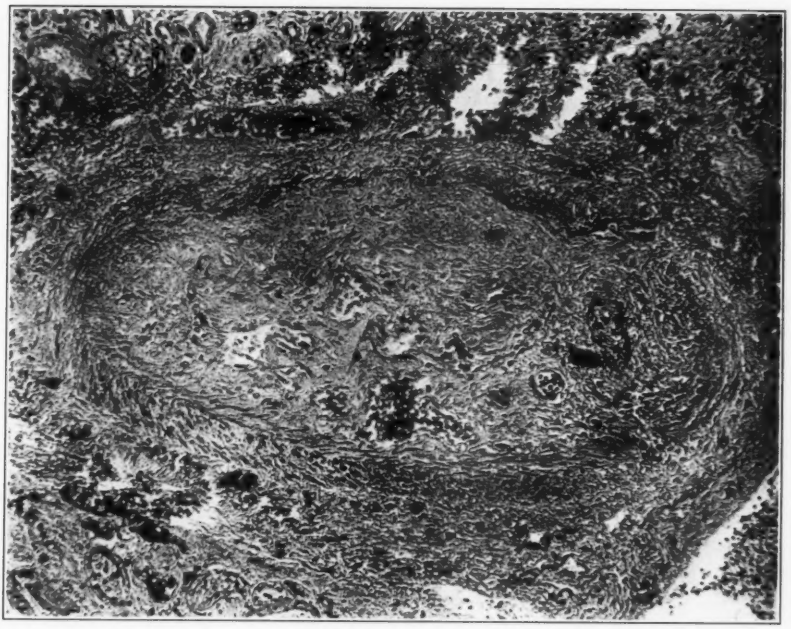
PLATE 20

FIG. 5. An advanced granulating lesion of an artery 1 mm. in diameter in the kidney. Most but not quite all the inflammatory cells have disappeared. Some of the smooth muscle of the media is preserved. Phosphotungstic acid hematoxylin stain.

FIG. 6. An almost healed lesion of an artery 1.5 mm. in diameter in the liver. Several foreign body giant cells are seen in the wall. Hematoxylin-eosin stain.



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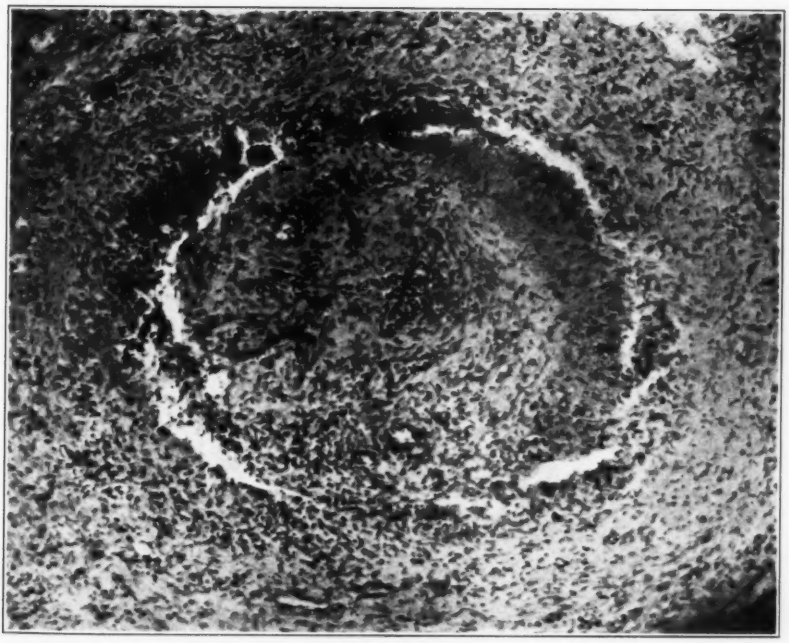

6

METASTASIZING FIBROLEIOMYOMA OF THE UTERUS *

Report of a Case and Review of the Literature

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Dissociation between clinical and histological malignancy in neoplasms reaches its acme in the "benign metastasizing tumors" in which the histological appearance is benign but which metastasize and prove to be clinically malignant. Among these tumors are certain chondromas, some angiomas, and "metastasizing adenomas" of the thyroid. To these must be added a few reported cases in which fibroleiomyomas of the uterus and myomas of other locations have metastasized. This report deals particularly with the problem of metastasizing fibroleiomyomas of the uterus.

As used in this paper, the term "metastasizing fibroleiomyoma" of the uterus refers to a tumor composed histologically, in both the primary growth and its metastases, of benign appearing, fully differentiated smooth muscle cells and dense connective tissue. This distinguishes it from primary leiomyosarcoma of the uterus and from sarcomas arising in fibromyomas or so-called sarcomatous degeneration of fibroids, in both of which the tumor cells have histological characteristics of malignancy in the primary tumor as well as in the metastases. Such unquestionable sarcomas lie beyond the scope of this study and will not be stressed. Instances are also recorded in the literature in which a uterine tumor was removed and called a fibromyoma after histological study, but in which local recurrences or metastases later showed histological evidence of anaplasia and malignancy. With such cases also we are not concerned in this report.

Metastasizing fibroleiomyomas of the uterus have been seen and described by several observers but their occurrence is so rare that some oncological authorities have never seen a case and have expressed doubts as to their existence. Thus Ewing [1] in discussing the possibility that uterine leiomyomas of benign appearance might metastasize states: "So far as I have been able to learn no

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case has been fully studied in which definite variations from the usual structure of leiomyoma were wanting, although in several instances these variations have not been very pronounced." Stout, in discussing the same problem, writes [2]: "While Jacquin has been able to collect 5 cases of tumors which morphologically were fibromyomas and yet metastasized and recurred always as unaltered myomas, such reports must be exceptionally rare and they leave one with the suspicion that there may be some change which has been overlooked even though the cases were reported by such keen observers as Langerhans, Minkowsky, Schlagenhaufer and von Franque." Meyer [3] and Albrecht [4] also believe that metastasis of mature myoma cells has not been proved up to the present. They believe that multiple sections from the primary tumors in the uterus in suspected cases would reveal a sarcoma in some portion. They fail, by this explanation, to account for the benign appearance of the structure in the metastases in these cases, and their lengthy discussions together with that of Raab [5] are beside the point.

Against these opinions stand the written words of a host of authorities who accept these tumors. Among them are Kaufmann,[6] Borst,[7] Aschoff,[8] Wolff,[9] Ribbert,[10] and others.

The question can then legitimately be asked whether or not such a tumor exists in the uterus or elsewhere. Since appeal to authority cannot settle this question, a critical evaluation of the cases reported in the literature, together with the new evidence obtained by study of a case, may, we hope, help clarify the subject. My attention was drawn to this problem after studying a case which seemed to fulfill the criteria for this type of neoplasm. The microscopic sections have been seen by about twenty pathologists who, suspecting that a benign myoma would not be submitted for pathological opinion, were nevertheless unable to make a diagnosis of malignancy.

Because of their rarity metastasizing fibroleiomyomas of the uterus are not of great practical importance. To the oncologist, however, they are of great interest because they illustrate certain limitations in the purely histological diagnosis of malignant disease, emphasize the inadequacy of the classical criteria for malignancy, as opposed to benignancy, remind him to be particularly cautious in the diagnosis of certain types of tumors, and

illustrate differences from accepted biological behavior in a number of interesting respects.

This paper deals with the case of a patient who exhibited the clinical picture of chronic pulmonary obstruction with cor pulmonale and polycythemia, and who died with right sided heart failure. The changes in the lungs during life suggested most strongly a chronic pulmonary infection. A symptomless pelvic mass was recognized during life but its relation to the changes in the chest was uncertain prior to autopsy. From a uterus the site of multiple tumors resembling fibroleiomyomas, massive metastases occurred to the lungs and to a tracheobronchial lymph node. The microscopic appearance of the metastases and of the uterine tumors was that commonly seen in uterine fibroids, the cells being benign in appearance and fully differentiated.

Report of Case

Clinical History: M. B., a housewife, aged 36 years and of English descent, was under observation at the University of Chicago Clinics (Unit #100,419) from March 12, 1934, until her death 15 days later. She gave a history of dyspnea, wheezing respirations, nocturnal headaches and nosebleeds during the preceding 18 months, a productive cough for about a year, marked orthopnea for 9 months and swelling of the ankles and abdomen for 6 months.

The patient had a prolonged chill accompanied by transient pain in the chest in May, 1933. During that summer she had a period of afternoon rise in temperature. She lost 77 pounds in weight between February and October, but had regained 25 pounds before her present hospital admission 5 months later. Her sputum was abundant, thick and yellowish, but not bloody. After study of X-ray films a physician had made a diagnosis of tuberculosis more than a year before her death, but no tubercle bacilli and no fungi could be found in the sputum. This diagnosis was abandoned by him in October, 1933, when X-ray films showed a slight recession instead of the expected advancement in the lung lesions. X-ray therapy to the chest was then followed by temporary subjective improvement. In January, 1934, the heart was found to be enlarged and she was decompensated, having dependent edema and an enlarged liver.

The patient stated that she had had mumps, chickenpox, measles and meningitis in childhood. She had never been pregnant. Prior to January, 1933, her menstrual periods had been regular at 30 day intervals and of 5 days duration. In January, 1933, she had a menstrual period accompanied by nosebleeds. The next periods were in May, June and July, 1933. There had been none since. Her husband was in good health. Her mother had died of diabetes at the age of 57 years.

On physical examination the patient appeared acutely ill with cardiac decompensation and advanced pulmonary disease. She was cyanotic, very dyspneic and preferred the sitting position. Examination of the eyegrounds showed tortuous vessels and an aneurysm of the left superior papillary artery.

The fingers were clubbed. Expansion of the chest, especially on the left, was diminished. Inspiration was short and expiration was prolonged. There was dullness to percussion with increased tactile fremitus over the base of the lungs. Coarse and fine moist râles, not removed by coughing, were heard in these regions. The border of the left side of the heart was 2 cm. beyond the midclavicular line. No murmurs were heard. The rhythm was normal but the rate was 110. The blood pressure was 130/80. An electrocardiogram showed a right axis deviation with inversion of T in leads II and III. The abdomen was greatly distended and there was some question as to whether it contained free or encysted fluid. The liver extended 5 cm. below the costal margin. The lower extremities showed only a slight edema. A hard tumor mass was palpable above the pubis to the left of the midline. On vaginal examination the cervix was found to be pulled up and a hard mass replaced the uterus.

The erythrocyte count was 5,680,000 and the hemoglobin was 101 per cent. The red cells showed a slight microcytosis and polychromatophilia. The leukocyte count varied between 6100 and 9200 with a differential count of 74 per cent neutrophils, 18 per cent lymphocytes, 6 per cent monocytes and 2 per cent basophils. The urine, except for a few white cells, was negative. No acid-fast bacilli or fungi were found in the thick sputum on repeated examination. The blood Wassermann and Kahn tests were negative. On attempted abdominal paracentesis a resistant mass was encountered and only a little bloody fluid which contained no unusual cells was obtained.

X-ray films made after admission showed many areas of increased density scattered throughout both lungs (Fig. 1). They were more marked in the lower lobes. There was a suggestion of bilateral cavitation. It was impossible to differentiate between tuberculosis and neoplasm from the films, although the picture was unlike that of the usual metastases to lungs. X-rays made by another laboratory in February, 1933 revealed an involvement of the lungs which already at that time resembled and was almost as spectacular as the condition shown on the last films.

The patient became more cyanotic and dyspneic, symptoms which were alleviated only by the use of the oxygen tent. The temperature varied between 100 and 102° F. and death occurred 15 days after admission to the hospital.

Postmortem Examination

The body was obese, weighing 187 pounds and measuring 162.5 cm. in length. The abdomen was protuberant with bulging in the flanks and a palpable mass was present in the midline extending upward to slightly above the level of the umbilicus. The legs and posterior parts of the body were edematous. The head and neck, the lower extremities and the back showed a marked cyanosis.

The pleural cavities had a few fibrous adhesions. The lungs were heavy and voluminous. They were everywhere studded with firm, pink to reddish tumor nodules varying in size from just visible to larger masses measuring up to 5 cm. in diameter. Intermingled with these solid masses were thin walled, semitranslucent

emphysematous sacs which showed the same variation in size. They collapsed on slight pressure. On forcing air into the lungs through the trachea these air cysts were reinflated, demonstrating their communication with the bronchial tree. They again collapsed when the pressure was released. Tumor nodules of both types, solid and cystic, projected above the surface of both lungs. A few tumors of each type were pedunculated, particularly along the lower lung margins (see Figs. 2, 3 and 4).

On the cut surfaces the solid tumors were found to be scattered throughout all lobes, but large air cysts were found only near the surface of the lung. The solid tumors were sharply demarcated but had no visible capsules. They were a reddish gray in color and the cut surface showed irregular whorls resembling a fibromyoma. Between these solid tumor nodules the parenchyma of the lung presented a honey-comb appearance because of small, thin walled air sacs. Most of the surface made by cutting was occupied by either solid tumor or cystic spaces so that there were only small, irregularly interspersed areas which appeared to be composed of functioning lung parenchyma. Frothy bloody fluid escaped from the surfaces. Each lung presented the same appearance. The right lung in addition contained a large firm mass measuring 13 by 12 by 12 cm. in the lower and lateral part of the upper lobe. This tumor contained a large cavity that was filled with a thick mucinous fluid which was sterile on bacterial culture. The walls of this cavity, while generally up to 2 cm. in thickness, showed portions as thin as 3 mm. Adjacent to this large tumor were several smaller tumors with mucinous material in their centers.

The tracheobronchial lymph nodes were slightly enlarged and several nodes showed areas of tumor tissue under the capsule resembling that seen in the lung.

The emptied heart weighed 475 gm. The increase in weight was due mainly to hypertrophy of the right ventricle, which, although the chamber was dilated, measured from 8 to 9 mm. in thickness. The left ventricle was normal in size and its wall measured 14 mm. The heart valves appeared normal. The myocardium showed no scarring and microscopically only a few small foci of mononuclear cell infiltration and a few small focal areas of fatty change were seen. The coronary arteries and aorta showed a

minimal amount of atheromatosis. The pulmonary artery and its main branches appeared normal. Their walls were thin and elastic.

The abdominal cavity contained about 600 cc. of a slightly turbid, yellowish brown fluid. The omentum was free. The intestines were cyanotic and were displaced upward by a mass arising in the pelvis from the uterus.

The liver weighed 1810 gm. Microscopically a marked hyperemia in the centers of the lobules with some necrosis in these areas, slight fibrosis, and marked fatty changes were present. The gall bladder and extrahepatic bile ducts appeared normal.

The spleen weighed 340 gm. It was cyanotic and firm and microscopically showed a diffuse fibrosis. Accessory spleens 1 and 2 cm. in diameter showed chronic passive congestion similar to that observed in the main spleen.

The gastric mucosa showed a diffuse hyperemia and numerous petechial hemorrhages. The duodenal mucosa was intensely hemorrhagic and exhibited a number of irregular erosions measuring up to 2 cm. in diameter. Throughout the remainder of the gastro-intestinal tract the walls showed variable amounts of edema, hyperemia and petechial mucosal hemorrhages.

The kidneys were cyanotic and showed also cloudy swelling with a moderate amount of granular degeneration of the epithelium of the convoluted tubules. The lower urinary tract appeared normal.

The uterus was enlarged, measuring 18 by 16 by 10 cm. (Fig. 5). Its upper surface was covered by smooth peritoneum. The uterine mass was nodular but fairly symmetrical except for nodules that projected into the right broad ligament. The surfaces made by cutting showed that it was composed of many firm nodules from a few mm. to 5 cm. in size, embedded in a fleshy fibrous stroma. The tumor nodules shelled out easily. Many of them were globular while others were elongated and irregular in contour. Closer examination showed that the tumor nodules occupied the anterior, lateral and upper walls of the uterus, and that the posterior myometrium was normally thin and muscular, measuring just over 1 cm. in thickness. The tumor nodules resembled fibroids, being firm, reddish gray, and composed of interlacing whorls of tissue. One tumor nodule showed an area of liquefaction necrosis in its center, and several others, including those

which projected into the broad ligament, were edematous. The veins in the right broad ligament were dilated and contained red ante mortem thrombi.

The uterine cavity was elongated and distorted. The endometrium was intact and normally thin, and except for a slight hyperemia was not unusual. The fallopian tubes were bilaterally thin, slender and of uniform caliber; their fimbriated ends were open and free. The ovaries were of normal appearance and on microscopic examination appeared to be functioning, having numerous ova and several small follicular cysts.

The thymus, adrenals, mammary glands, pancreas and pituitary appeared normal. The thyroid microscopically showed a diffuse and focal dense connective tissue increase with hyalinization in some places and lymphocytic foci in others. The brain showed only an anomaly in the circle of Willis. Instead of a single anterior communicating artery joining the anterior cerebral arteries there was a network of arteries from which passed a single right anterior cerebral and two left anterior cerebral arteries, the latter communicating 1 cm. from their origin but still remaining separate trunks. The lymph nodes showed no changes except those mentioned with respect to the tracheobronchial nodes. The muscular system and skeleton likewise showed no abnormality. The bone marrow from a rib and from a lumbar vertebral body showed a hyperplasia of hemopoietic elements.

Anatomical Diagnoses: Multiple fibroleiomyomas of the uterus; multiple solid and cystic fibroleiomyomatous metastases to each lung and to the tracheobronchial lymph nodes; pulmonary emphysema and slight hyperemia; hypertrophy and dilatation of the heart, particularly of the right ventricle; generalized passive congestion; ascites; edema of the dependent parts of the body; parenchymatous degeneration of the liver and kidneys; petechial hemorrhages in the mucosa of the stomach, jejunum, ileum and colon; acute erosions of the duodenal mucosa; fibrous pleuritis; accessory spleens (2); and anomaly of the circle of Willis.

Essential Microscopic Features

Except for the tumors in the uterus, lungs and tracheobronchial lymph nodes, the microscopic changes have been briefly given and will not be repeated. Tissues were fixed in Zenker's and

Bouin's solutions and in formalin. Embedding was in paraffin and celloidin. Sections were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin, Mallory's aniline blue stain for connective tissue, Van Gieson's connective tissue stain and Masson's trichrome method. The distribution and fate of the elastic tissue in the lung was studied by Weigert's method. Frozen sections of heart, liver and kidney were stained for fat. The autopsy was begun 3 hours and 20 minutes after death but all tissues were not fixed immediately so that the results of the differential staining were not perfect.

Uterus: Sections from sixteen locations in the uterine tumors show, except for minor differences, the same microscopic picture. The tumors resemble the common fibroleiomyoma with the smooth muscle component conspicuous (Fig. 9). In some areas the tumor is very vascular and in these places the cells tend to be shorter than elsewhere and to exhibit a tendency to a perivascular arrangement. Dense connective tissue stroma with much collagen is abundant in some nodules, while others show much edema fluid separating the tumor cells. Other degenerative changes, common in fibroids, except for the liquefaction necrosis seen in one tumor nodule, are absent. In one section made from the tumor which extended into the broad ligament superficial infiltration of tumor cells into skeletal muscle is noted although this had not been observed on gross dissection. Invasion of tumor cells through the wall of a uterine vein to form a sessile mass projecting into the circulating blood is seen in one section.

The predominant cytological constituent of the tumor is smooth muscle. Anaplasia is absent in the tumor cells. The nuclei are regular, symmetrical and cigar-shaped, and lie parallel. There are more nuclei per unit volume than are usually seen in proliferating tissues composed of young connective tissue, and the nuclei contain more chromatin than do young fibroblasts. Mitotic figures are rarely found. The cells form fascicles which interweave. Among the muscle cells are connective tissue cells, which are numerous in some areas. Mast cells are also scattered throughout the sections.

The stains for fibrillar cytoplasmic material show that there are myofibrils within tumor cells. They can be seen where the cells are cut transversely and they also appear where the cells are

cut longitudinally, although here their localization within the cytoplasm of the cells is less certain. The myofibrils, in many places, appear to be degenerating, being clumped near the nucleus and taking a reddish brown instead of a blue color with the phosphotungstic acid hematoxylin stain. Coarser myoglia fibrils, which stain blue to black with the same stain, are visible on the surfaces of the cells in many regions.

The chromatic properties of the tumor cells in the various stains, with respect to both the nucleus and the cytoplasm, are best compared with the blood vessel walls in the same sections. They are seen to resemble the smooth muscle cells of the tunica media and not the young fibroblasts of the tunica adventitia. The fibrils of the tumor cells have the tinctorial qualities of the elastic fibers of the blood vessel wall, where such are visible. Dense connective tissue with collagen is present in all sections. In some regions it is abundant while in the younger, perivascular regions very little is seen. The relationship and relative amounts of the various types of fibrils are easily seen in edematous regions where the cells have been teased far apart and conform essentially to the above descriptions.

Sections through the endometrium show an infiltration by plasma cells, lymphocytes and neutrophils, numerous thin walled blood vessels and an atrophic epithelium. The cervical glands are also atrophic. Numerous sections through the para-uterine vessels reveal many veins and one artery to be thrombosed by recent or by organizing thrombi not containing tumor at the levels studied.

Lung: Except for less vascularity and an even more highly differentiated cell morphology, the cytological appearance of the lung metastases resembles that seen in the uterine tumors. The essential tumor cells appear to be smooth muscle with a stroma of varying amounts of dense connective tissue. These cells form circumscribed but encapsulated infiltrative nodules, mainly in the interstitial regions, but also in the region of perivascular and peribronchial lymphatic channels (Figs. 6 and 7). Some nodules are composed of solid masses of tumor cells, but in the majority are also found irregular spaces lined by flattened or cuboidal epithelium resembling that seen in chronically scarred lung tissue. These spaces resemble persistent alveoli which have become lined

by cuboidal epithelium. Most of these alveoli are collapsed, with opposing epitheliums in contact, but others are open and appear to contain either air or mucus. The nature of the remarkable air cysts described in gross is revealed by studying the forms these alveoli assume. The cystic tumors appear to represent enormously enlarged alveolar sacs. Their walls are composed of smooth muscle tumor cells which are greatly elongated and they are lined by flattened epithelial cells. Solid and cystic lung tumors, therefore, differ only in whether an enlarging alveolus persisted within the tumor nodule. The large mucus-filled tumor mass found in the right lung has lining cells which appear to have formed the mucus. The presence of myofibrils and myoglia fibers can be demonstrated in the lung metastases (Fig. 8). No tumor growth is noted within large blood vessels in the lung.

Lymph Nodes: Fibroleiomyomatous tissue is seen in the peripheral sinus and in an afferent lymphatic of a lymph node removed from the region of the bifurcation of the trachea (Fig. 10). The cells here also appear fully differentiated and benign. The specific fibrils for smooth muscle are present. Fibrous stroma is present and the tumor tissue is relatively avascular.

Discussion

Several points in the clinical history are worthy of emphasis. Very striking was the clinical picture, which was that of pulmonary disease with obstruction to blood flow through the lungs, rather than that of pelvic neoplasm. The polycythemia, together with hypertrophy of the right ventricle, was effective in maintaining compensation for about a year, but the last 6 months of life were characterized by a progressive failure of the right side of the heart.

The long duration of the disease is also interesting and indicates that the rate of growth of the lung tumor metastases was slow, a fatal termination occurring after 18 months of illness. Since there was no great change in the X-ray films during the last year of life it is probable that the lung metastases had been present for a long time before they had attained a size and number sufficient to produce the first symptoms. The microscopic appearance of the tumor is likewise that commonly associated with slow growth.

Another striking feature was the marked disproportion between

the extent of the disease as indicated by the X-ray studies of the lungs and the degree of incapacitation of the patient. Her general condition was better than would be thought possible, and the rate of decline was slower than that accompanying the commoner lung infections or metastatic tumor to the lungs in which the X-ray signs are so advanced. The absence of regression of the tumor tissue in the lungs under X-ray therapy is in conformity with what is known about the radiosensitivity of this type of tumor.

The age of the patient is also interesting. Most uterine sarcomas occur near the menopause, while this patient was only 36 years of age.

Williams [11] was among the first to show that malignant uterine tumors may be derived from smooth muscle. Mallory [12, 13] gave staining procedures by which muscle cell tumors can be identified on the basis of their fibrils. Characteristic fibrils are present in this tumor so there is no question as to its fundamental myomatous nature.

It is generally recognized that there is no sharp point of differentiation between benign and malignant muscle cell tumors. The myosarcomas in general have less stroma and are therefore softer and fleshier. They tend to be more vascular and are unencapsulated and poorly demarcated. They are cellular, with changes in the nuclei varying from little change to extreme anaplasia. In general the nuclei are shorter, more chromatic, uneven in size and shape, and show an increased number of mitotic figures. The loss of normal nuclear structure is accompanied by loss of the characteristic cytoplasmic fibrils. Anaplasia may be so marked that the fundamental nature of a tumor, whether it originated from muscle cells or fibrous stroma, cannot be determined.

In the routine microscopic examination of surgically removed uterine fibroids not infrequently cellular tumors exhibiting some of the cytological features associated with malignancy mentioned above are encountered. In the recorded literature this incidence varies from a small fraction of 1 per cent to over 10 per cent. Corscaden and Stout have recently discussed this situation [14] and have pointed out the error in diagnosis by those who find the higher figures. In these tumors the cytological changes are sometimes misleading since in a series of untreated fibroids, or in a series treated lightly by X-rays, no corresponding high incidence

of fatal uterine sarcoma is found. Uterine sarcomas in general autopsy series are relatively uncommon. Kimbrough [15] found that the 5 year survivals were three times as great in so-called sarcomatous degeneration of fibroids as in the primary uterine sarcomas. Any errors in diagnosis would most likely have been in the first group.

Although it is a common experience to find suspiciously sarcomatous regions in uterine fibroids, which are clinically permanently benign, the case here reported is an example of a much rarer condition in which a tumor composed of benign appearing cells proved to be clinically malignant.

Since the usual cytological changes are frequently misleading in the diagnosis of malignant uterine tumors, more reliable criteria have been sought. The mitotic figure count as an index of malignancy is accepted as of value by many. Evans [16] in 1920 pointed out that the clinical malignancy closely follows the number of mitotic figures. Kimbrough,[15] Handley and Howkins,[17] and others, have found this procedure of value. Meigs [18] states that malignancy must be suspected if more than 1 or 2 mitotic figures are seen in 10 to 25 high power fields. Casey [19] has studied the number of mitotic figures per 1000 tumor cells in a variety of neoplasms and has found that in general benign tumors have fewer than 4 and malignant tumors more than 4 mitotic figures per 1000 tumor cells. In this respect also the tumor in our case is unusual in that the number of mitotic figures is not correlated with the clinical malignancy. In the uterine tumors (2000 cells), lung metastases (1000 cells), and lymph node metastases (1000 cells) no mitotic figures were encountered (autopsy material). Accordingly, by this criterion, this case falls into the group of benign tumors, and this is in agreement with its general microscopic morphology. Yet its known clinical behavior was that of a malignant tumor.

The routes of metastasis in this case were undoubtedly by the veins from the uterus to the lungs and through lymphatics from the lungs to the tracheal lymph node. Several theories have been proposed to explain how cells so harmless appearing might metastasize. Thus Proper and Simpson [20] state: "They are probably really benign and metastasize as the result of the cells of a leiomyoma penetrating a blood vessel and producing another

tumor at the place where these cells become lodged." Van Rijssel [21] and Sitzenfrey [22] believe that benign appearing tumor cells enter blood vessels by pressure atrophy of the vessel wall.

Nature of the Tumor: Grossly there was little to indicate that the tumors in the uterus were malignant or capable of producing metastases. The appearance was that of a uterus enlarged by common fibroids. The tumors were firm, reddish gray, silky and whorled in most places, and on the whole shelled out readily. Nowhere in the uterine tumors or in the metastases was the tumor tissue friable or fleshy like a sarcoma. In retrospect one feature in the gross appearance may be of the greatest importance in recognizing this type of tumor. Although the uterine tumors separate easily from the tissue in which they are embedded, *some of them are seen to be not globular but elongated and to have a little tendency to interweaving or intertwining of adjacent tumors — in other words, a plexiform growth.* In this respect it resembles the case of Lahm.[23]

Microscopically, although the appearance was that of fibroleiomyoma, two points in examination of the uterine sections reveal the possibility of metastases. The tumor cells were capable of invading veins and they were able to infiltrate striated muscle. Neither of these features had been noted grossly. They indicate an unexpected potentiality of the cells and clarify the mechanism of the metastases.

Microscopic sections from this case have been seen by about twenty experienced pathologists. A few of them commented on the unusual degree of vascularity of some, but not all, areas in the uterine tumors. Others said that this was of a degree not uncommon in fibroids. Small blood vessels are numerous in some places and the tumor cells immediately adjacent appear more immature than those at a distance. This vascularity is less striking in the lung metastases, being entirely absent in large portions, and it is not seen at all in the lymph node metastasis. So it appears not to be an essential part of the growth.

Could the tumor be one of a primary vascular growth? The fact that the tumor cells immediately about blood vessels are shorter, appear younger, and that there is less collagen speaks for more rapid growth in these regions. The tumor cannot be proved to have a vascular origin. In fact, the presence of adventitial cells

separating the media of the blood vessels from tumor cells in certain areas is evidence against such a hypothesis. Furthermore, a perivascular proliferation of tumor cells is no proof of a vascular origin, but it may rather indicate a more favorable environment for growth in the form of better nutrition, a feature commonly seen in carcinomas and other tumors.

Discussion of Nomenclature: I have no strong convictions about the nomenclature of this type of tumor and have used the descriptive term "metastasizing fibroleiomyoma" at the insistence of some oncologists who saw the sections, and to strengthen the teaching points of the case. I have refrained from using the word benign. The incongruity of the term is apparent and I appreciate that the clinical outcome of the case resembled that of a malignant tumor.

In the nomenclature of malignant mesenchymal uterine tumors, by usage the term "sarcoma," with some qualifying term such as leio-, fibro-, mixed cell, undifferentiated, and so on, is widely accepted. This is true aside from the question of their primary origin from myometrium or from a previous fibromyoma. The term "myoma malignum" has been used by some writers for tumors in which the microscopic appearance suggested a malignant tumor but in which definite proof of malignancy was wanting, a position not infrequently met with in the routine examination of surgically removed fibroids. This same term is also used by others in a different sense to describe those instances in which a fibroleiomyoma becomes frankly malignant. The term "sarcoma myomatoides" has an even more diversified usage, having been used to describe tumors of each of the above three types. So none of these terms is entirely applicable to this case. Strong [24] has reviewed the problem of nomenclature in considerable detail.

Casting aside for the moment the term I have used, what name would be most suitable? I do not say that this is not a sarcoma, but if it is, that diagnosis must be made on other grounds than tissue morphology. Shall we say sarcoma of the uterus, as did Jacquin,[25] even if the histological appearance is benign? To do this is an admission that the diagnosis of sarcoma from biopsy material before the final clinical outcome is known is impossible in some instances.

As broad criteria of malignancy the familiar qualities of neoplasia, heterotopia and anaplasia present themselves. Of neoplasia

there can be no question in this case, but it is a property shared by benign and malignant tumors alike. Heterotopia was also present but there was no evidence of anaplasia. Under these circumstances the term myoma malignum might be considered more appropriate for this case than metastasizing fibroid, except that, as pointed out, this term is used by some in instances where cell anaplasia is present. In dealing with epithelial tumors the term "adenoma malignum" is used by some when there is neoplasia, a minimal degree of anaplasia, and no proof of heterotopia. It is useful in transmitting from the pathologist to the clinician the idea that the tumor is probably malignant but not so proved. In the present case the tumor was clinically malignant because of heterotopic muscle cells. McFarland [26] concludes that metastasis is the only proof of malignancy in dealing with muscle tumors. But benign fibroleiomyoma cells are capable of growing ectopically under special circumstances as shown by Brewer's [27] case in which cells from uterine fibroids, implanted into the abdominal wall, grew.

The problem as to nomenclature is not an important one since the question does not often arise.

Review of the Literature

A. Metastasizing Fibromyomas of the Uterus: A critical comparison of our case with those previously reported is extremely difficult because the earlier papers are not illustrated or are inadequately illustrated, histological descriptions are absent or sketchy, and there is considerable confusion in the nomenclature. Undoubtedly other cases have been overlooked, but the following 4 cases summarize those found in a fairly careful survey of the literature. Some of these cases are without description or illustration.

The 1st case reported was that of Krische [28] in 1889, in which fibromyomas of the uterus metastasized to many parts of the body. There was said to be abundant fibrous stroma. Orth [29] accepts this case as one of metastasizing fibroids without comment. Meyer,[3] however, refuses to accept this case, stating that its sarcomatous nature is firmly established.

The 2nd case of this type was reported by Langerhans [30] in 1893. It was that of a 60 year old woman who had multiple tumor

nodules in the uterus. One of these was predominantly smooth muscle, another was pure smooth muscle and the rest were fibromyomas. There were multiple pulmonary metastases composed of cells which were larger and more irregular than those in the uterus. There was, therefore, some change from normal smooth muscle structure (anaplasia) in the lung, the degree of which cannot be estimated because the paper is not illustrated. He called it a "myoma laevicellulare malignum."

The 3rd case on record is that of Minkowski,[31] who in 1901 at autopsy found metastases composed of adult smooth muscle but lacking all stroma, in the lungs, liver and muscles of a 43 year old woman who 2 years before had had a complete hysterectomy for a "fibromyoma" of the uterus. There are no illustrations or detailed descriptions, the case having been presented briefly before the medical society at Köln. He gave it the name of metastatic myoma.

The 4th case was that which Schlagenhaufer [32] reported in 1902. A woman, 58 years of age, had multiple uterine tumors with metastases to the liver and lungs. Microscopically part of the uterine masses were typical fibromyoma while others were composed of muscle cells exclusively and had many large and small blood vessels, giving a cavernous appearance. There was no fibrous stroma. Some of the muscle cells showed anaplasia, being large and having multiple nuclei. In the metastases the vascular cavernous structure was not seen. The drawing which accompanied this report illustrates pure smooth muscle of a well differentiated type. He called his case "myoma telangiectodes."

Of these cases those of Langerhans and Schlagenhaufer can be discarded from this group, since, by their own descriptions, the tumor cells showed a certain degree of anaplasia. Schlagenhaufer was impressed by the vascularity of his tumor. His paper, alone, is illustrated. Minkowsky and Schlagenhaufer thought that the absence of stroma (binding-substance) explained the malignant behavior of their tumors. Only the case of Krische had stroma.

In addition to these case reports of benign appearing metastasizing tumors of the uterus the literature contains references to such occurrences without presenting the details. Thus, Lockyer [33] states: "In common with other observers, I have examined the metastatic deposits from the lungs and pelvic cellular tissues of a

case in which there was a large myoma of the uterus, and where all the growths, primary and secondary, had the structure of an innocent-looking myoma. Unfortunately the specimen, which was removed by the late Stanley Boyd, had been lost, but the sections are preserved, and the lesson they teach suffices to obliterate any suspicion I may have had as to such a condition being a fact."

Also McFarland [26] in speaking of myomas in general, including those from the uterus, states, without giving details of such cases: "Histopathologic prognosis is fraught with difficulty. We have all seen, on the one hand, the tumor whose histological structure appeared to be that of an ordinary fibroid, accompanied by one or more metastases of identical appearance, and on the other, a tumor of the most suspicious appearance whose history has terminated with its removal."

Kaufmann,[6] also without citing a case, writes: "Malignant myomas produce metastases by the blood channels, possibly through the lymphatics also; therefore biologically, according to their growth, they are malignant, but histologically they are typical myomas, distinguished from sarcomatous myomas in that their muscle cells are more uniform and of higher development."

Not available to the writer is the volume in which Johnstone (according to Lockyer [34]) mentions 10 cases in which pulmonary and other metastases showed a structure of unstriped, benign appearing muscle tissue.

B. Recurring Fibromyomas of the Uterus: In discussions of this subject, the cases which von Franqué [35] reported in 1907 and which Jacquin [25] described in 1921 are usually included. They are examples, not of metastasizing but of recurring fibroleiomyomas. The case of Jacquin showed a microscopically benign recurrence 10 months after a hysterectomy for benign fibromyoma. The patient of von Franqué was even more interesting because of three recurrences over a period of 7 years. The tumors showed pure myoma with very little connective tissue, and areas of edema and of hyaline degeneration. The muscle of the vessel walls was not demarcated from tumor in some places.

More recently Neugebauer [36] in 1927 briefly reported an instance in which 6 years after supravaginal hysterectomy for benign myoma there were found three pelvic tumors entirely

separated from the amputation area and all showing the histological structures of simple benign myoma. This paper is without illustration or detailed histological report.

Christophorakos [37] described the case of a fibromyoma of the uterus in which there were three recurrences in 3 years, the first two of which appeared like benign myomas, while the third showed areas of pleomorphic sarcoma in addition to myoma. Matthews and Stier [38] saw a patient who died of a fourth recurrence and distant metastases about 10 years after a uterus with multiple fibromyomas was removed. The recurrences showed progressive deviation from normal, the first recurrence appearing benign and the last like a spindle celled sarcoma.

These cases of Christophorakos and of Matthews and Stier raise the question as to whether the other foregoing tumors would have changed their morphology to frank sarcoma if they had recurred again several times.

C. Intravascular Uterine Fibromyomas: In addition to the groups of metastasizing fibromyomas and recurring fibromyomas mentioned above, a third group of cases sheds light on this subject. This includes cases in which uterine fibromyomas extended into the regional blood or lymph vessels, yet failed to metastasize. They are of special interest in this connection because they may be illustrations of the early stages of the type of metastasis seen in the case here reported. Birch-Hirschfeld [39] (3 cases) and Meyer [40] described tumors with intralymphatic extensions. Von Franqué,[35] Hörmann,[41] Dürck,[42] van Rijssel [21] (2 cases), Seyler,[43] and Sitzenfrey [22] (3 cases) reported cases with intravenous growth. Lahm's [23] case was unique in showing both lymphatic and venous growth, although he saw changes at least suspicious of malignancy.

In this connection the report of Frank [44] should be kept in mind. He described 3 cases of plexiform growth within the lymphatics of the myometrium by a tissue which resembled endometrial stroma.

Summary

A case is reported in which multiple tumor metastases to the lungs from the uterus led to chronic obstruction to blood flow through the lungs, polycythemia, enlargement of the right side of the heart, and finally to heart failure. The uterine tumors micro-

scopically appeared to be benign fibromyomas with areas that were quite vascular. The lung and tracheal lymph node metastases appeared even more mature and benign. In the absence of changes in color and consistence, local invasion of veins or rapid growth, uterine tumors which resemble fibroids but have a little tendency to plexiform growth should be considered potentially malignant. No new microscopic criteria have been discovered by which the malignancy of such cases can be recognized in the future. The mitotic figure count on postmortem specimens was non-informing as to the malignancy. The tumor in our case was histologically benign appearing, but clinically malignant. Such cases are much rarer than those in which histologically malignant appearing uterine tumors (cellular myomas) prove to be clinically benign. Four cases of metastasizing uterine fibromyomas reported previously, 2 of which, however, showed signs of malignancy, are briefly summarized.

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DESCRIPTION OF PLATES

PLATE 21

FIG. 1. X-ray of chest made about a week before death. A similar degree of involvement was present a year earlier. The picture is not like that usually produced by tumor metastases to the lungs.

FIG. 2. X-ray of the excised inflated lungs. The solid and the cystic nodules can both be seen, the latter particularly at the margin. The heart is still attached. The large tumor metastasis is in the right lung.

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Steiner Metastasizing Fibroleiomyoma of Uterus

PLATE 22

FIG. 3. Posterior view of the lungs. The largest tumor mass is in the right lung. Pedunculated metastases can be seen at the margin.

FIG. 4. Anterior view of the lungs. Some tumor nodules are solid and others are air-containing with walls containing fibromyomatous tissue.

FIG. 5. Uterus. From below upward are seen the vagina, cervical canal and uterine cavity. Numerous tumor nodules occupy the anterior and upper uterine walls, and several nodules extend out into the right broad ligament.

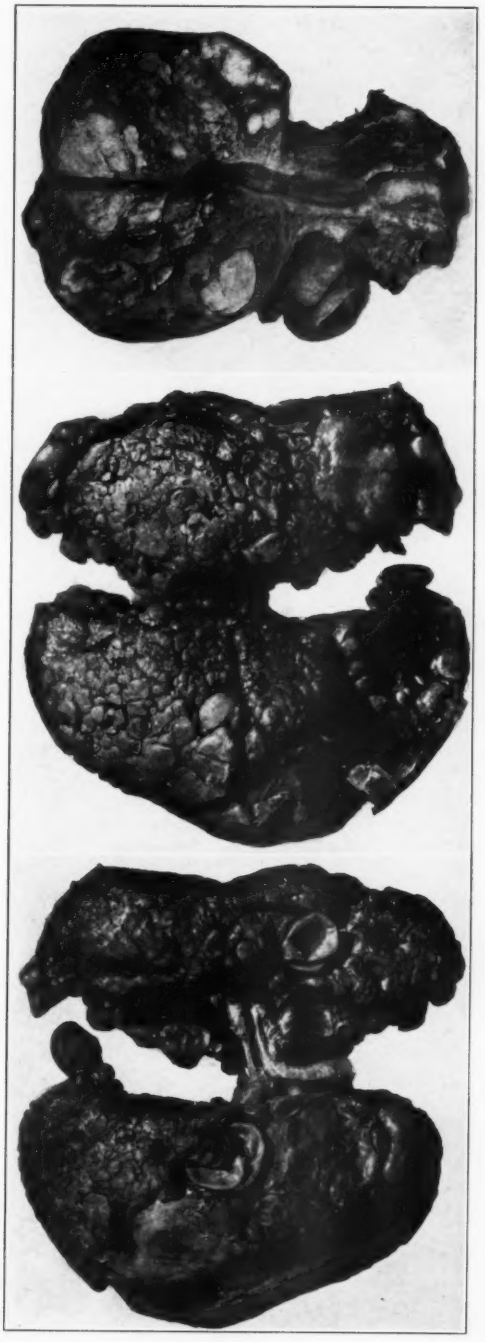

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PLATE 23

FIG. 6. Section showing metastases to lung. The walls of the cystic tumor nodules are composed of smooth muscle lined by epithelium. A small, solid tumor metastasis is also shown. Only small areas of aerated lung resembling that illustrated are present in both lungs. × 10.

FIG. 7. Section showing the myomatous nature of the metastases to the lung. Hematoxylin-eosin stain. × 650.

FIG. 8. Section of metastasis to lung illustrating myoglia fibrils and myofibrils in cross and longitudinal section. Phosphotungstic acid hematoxylin stain. × 2600.

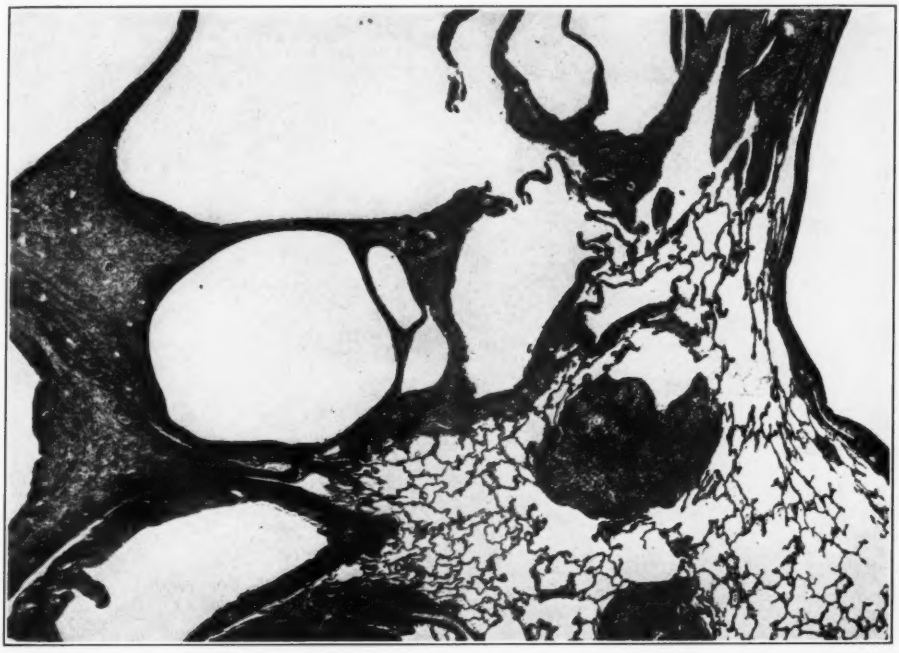

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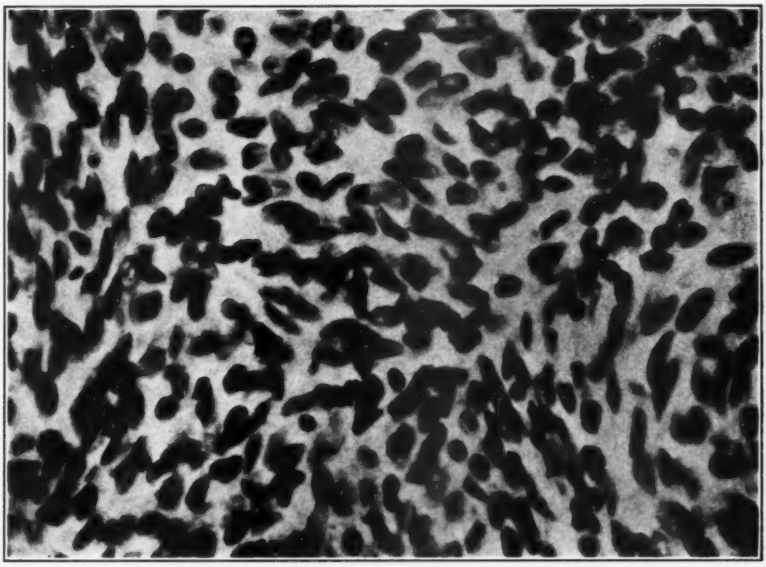
PLATE 24

FIG. 9. Section of a cellular area in the uterine tumor. Many of the cells are transected. Areas more suspicious than this were not seen. Hematoxylin-eosin stain. × 700.

FIG. 10. Metastasis in the peripheral sinus of a tracheal lymph node. The fully differentiated myomatous nature of the tumor is easily seen. × 550.



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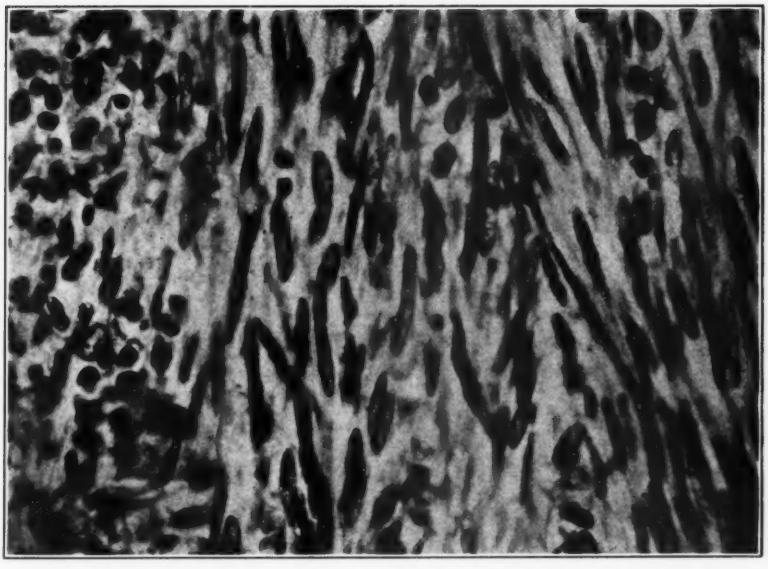

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Steiner Metastasizing Fibroleiomyoma of Uterus

PRIMARY HYPERPARATHYROIDISM WITH EXTENSIVE RENAL CALCIFICATION AND SECONDARY HYPERPLASIA OF THE PARATHYROIDS *

Report of a Case

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That hyperparathyroidism may produce renal insufficiency through renal calcinosis and calculus formation, and that renal insufficiency of varied etiology may be associated with hyperplasia of the parathyroid glands is now well established. Albright and Bloomberg [1] conclude that hyperparathyroidism is such a frequent cause of renal stone formation that its presence must be ruled in or out in every case of this disease and cite 11 cases out of 23 proved cases of hyperparathyroidism where the presence of renal stones was the only clue that led to the diagnosis of the underlying condition. Ettinger and Magendantz [2] reported a case with decreased urea clearance and phenolsulphonephthalein excretion but uncomplicated by nitrogen retention, where evidence of extensive calcification of the kidneys was immediately evident on X-ray examination. In discussing the renal complications of hyperparathyroidism, Albright, Baird, Cope and Bloomberg [3] consider the renal lesions as divisible into three groups: (1) those (type I) associated with a pyelonephritis secondary to the formation of calcium phosphate stones in the renal pelvis; (2) those (type III) presenting calcium deposits in the kidney parenchyma as well as in other organs, but without chronic renal change and associated with acute parathyroid poisoning with anuria and death from undetermined cause in a few days; and (3) a third group (type II) differing from type I in that the calcium deposits are in the parenchyma of the kidney, which may be the only organ involved, rather than in the kidney pelvis, and from type III in that the kidneys show long standing changes simulating both chronic glomerular and vascular nephritis.

Over 30 years ago MacCallum [4] first reported parathyroid en-

* Received for publication June 17, 1938.

largement associated with nephritis. Bergstrand [5] in 1921 more particularly called attention to the relation between renal function and parathyroid enlargement. More recently sufficient evidence has been accumulated from experiments and autopsies to conclude that parathyroid enlargement is a relatively constant finding in renal insufficiency. Thus, Pappenheimer and Wilens [6] conclude from an analysis of the weight of the parathyroids in 27 nephritics and 72 miscellaneous cases showing normal kidneys that the mean weight of the parathyroids in various types of chronic renal disease exceeds that of the non-nephritic cases by from 50 to more than 100 per cent, that usually three or four of the glands share in the enlargement, and the weight increase is roughly proportional to the severity and extent of the renal lesions and to the intensity of the clinical signs of renal insufficiency. Castleman and Mallory [7] report 29 cases of "secondary" hyperplasia associated with chronic renal insufficiency of various grades, offering certain histological criteria they consider specific of secondary hyperplasia. Their 29 cases presented varying degrees of enlargement of the parathyroids and even when the enlargement was limited to a single gland, histological examination always revealed evident hyperplasia in the other glands also. Furthermore, Jarrett, Peters and Pappenheimer,[8] and Pappenheimer [9] have shown in the rat that reduction of functional renal substance leads to a decided increase in size of the parathyroids.

In the light of these observations we wish to present for consideration a case * we believe may be interpreted as one of classical primary hyperparathyroidism associated with extensive calcification of a horseshoe kidney (Albright's type II). Removal of the parathyroid tumor was followed by dramatic improvement, a return to normal of the blood chemistry, and healing of the bone lesions. The extensive renal calcification persisted, however, and over the space of a few years hypertension and marked renal insufficiency developed, resulting in enlargement of the remaining three parathyroid glands and death in uremia.

* The case is 1 of 4 reported by Gutman, Swenson and Parsons [10] in 1934 (Case 1) and their article is illustrated by X-rays of bones and of the abdomen demonstrating calcification of the horseshoe kidney. It includes a table of balance studies. Certain of the chemical data have been further reported by Gutman, Tyson and Gutman [11] in 1936 (Case 1). However, we shall include a very brief clinical summary and complete the clinical and chemical data.

Report of Case

Clinical History *: A 34 year old American salesman was first seen at the Presbyterian Hospital in July, 1933, complaining of pain in the right hip for 9 months and of increasing weakness for about 2 years. There was no family history of disease of the bones or childhood history of rickets or late walking. For the past 2 to 3 years he had been losing weight and strength and had noted thirst and polyuria with nocturia (3 to 4 times). There was no history of hematuria, oliguria, dysuria, hypertension, acute nephritis, sore throat, tonsillitis or scarlet fever.

One year before entry the patient fractured his right humerus when throwing a ball. There was no history of direct trauma. X-ray studies revealed a cyst in the region of the fracture. The remainder of the skeleton was not examined. The fracture healed in 8 weeks, the patient being given calcium, cod liver oil, and ultraviolet therapy. During this time he lost 30 pounds. He was given iron intramuscularly, liver extract, and iron by mouth, and treatment with cod liver oil and calcium lactate, supplemented by viosterol, was continued. His general health improved and he gained 20 pounds and was able to return to work.

Six months before entry his urine was said to have contained albumin. About this time slight trauma to the right shin was followed by swelling, not especially tender or painful, which on subsiding in about 2 weeks left a hard, smooth, irregular bony prominence that persisted unchanged up to the time of admission.

Three months prior to admission an X-ray of the hip because of pain in that region revealed the presence of three cysts and the patient found that he required a hat ¼ size larger than he had worn during the previous 14 years. He had lost 30 pounds. About this time he began to vomit 3 to 4 times per week, usually in the morning. Although nausea and occasionally vomiting was associated with the cod liver oil, he continued taking it with calcium lactate after finding that omission resulted in exacerbation of his symptoms.

On admission to the hospital physical examination revealed: temperature 99.4, pulse 88, respiration 22, blood pressure 150/90 mm. Hg. The heart was not enlarged. The urine showed a very faint to faint trace of albumin but no Bence-Jones protein. The specific gravity was 1.006–1.012 and glucose 0. Microscopic examination on five occasions was negative; twice there were reported white blood cells, and once "casts and crystals." The Wassermann reaction was negative. The red blood cells were 3,760,000, and the white blood cells 15,200. A differential count was normal. The serum calcium was 15.7 mg. per cent, phosphorus 2.6 mg. per cent, phosphatase 33.9 Bodansky units per cent, serum protein 6.5 mg. per cent, and the blood urea nitrogen 35 mg. per cent. Roentgenograms revealed that practically all the bones contained multilocular cystic areas of decreased density. The skull presented a characteristic finely granular appearance, and there was seen a horseshoe kidney containing many areas of calcification. Metabolic studies showed definitely increased urinary excretion of calcium and phosphorus. After balance studies the patient was kept on a high calcium diet.

* I am indebted to Dr. A. B. Gutman and to Dr. W. B. Parsons for the clinical data and permission to report this case, and to Dr. A. M. Pappenheimer for help in the preparation of this material.

He was operated upon on Aug. 17, 1933, and an adenoma of the right lower parathyroid was discovered. The gland was removed and was found to weigh 3.5 gm. Gross and histological descriptions of this adenoma will be found below with the description of the remaining three parathyroids. Following operation he had slight transient hypoparathyroidism, readily controlled with

TABLE I

Data on Blood Pressure and Blood Chemistry

Date	Blood Pressure	Calcium	Phosphorus	Phosphatase	Non-protein nitrogen
	mm. Hg.	*mg. %*	*mg. %*	*mg. %*	*mg. %*
7/10/33	150/90				
7/11/33	135/90				
7/14/33		15.7	2.7	34.9	Urea 35
7/15/33	134/80				
7/24/33	130/90				
7/29/33	130/90				
8/1/33		15.4	2.6	34.8	39
8/15/33		15.8	3.1	28.3	40
8/17/33	* 118/–				
	* 105/–				
	* 165/–				
	* 135/–				
8/18/33		9.7	2.4	32.1	47
8/20/33		9.2		27.9	39
8/21/33		8.8	1.9	29.9	
8/23/33		8.7	2.1	36.2	25
8/28/33		8.1	2.7	45.6	
10/6/33		9.4	4.2	16.6	51
12/29/33		10.5	4.0	7.9	60
3/16/34		10.4	4.0	6.1	60
8/7/34		10.2	3.0	4.2	44
7/23/35		10.3	2.9	4.5	50
1/26/37		9.7	3.5	4.2	54
6/22/37	210/120	9.8	3.3	4.6	66
9/20/37	200/120	9.2	3.3	5.4	79
2/9/38	240/150	10.5	6.0	5.4	110
2/10/38					107
2/12/38	240/130	9.6	5.2	4.4	107
2/15/38					95
2/18/38	170/110	9.2	4.6	3.8	125

* Systolic pressures at start and at 10 minute intervals during operation for removal of the parathyroid tumor.

calcium lactate. The blood calcium returned to normal and remained normal, and over a period of months the serum phosphatase approached normal. The bone lesions healed and the patient improved and returned to work. He was followed intermittently in the out patient department and was seen again 4 years after operation complaining of loss of weight, occipital headache and nocturia. The blood pressure was 210/120 mm. Hg. The heart was slightly enlarged to the left, the apex impulse not diffuse or heaving. The non-protein nitrogen was 66 mg. per cent. Three months later the blood pressure was

200/120, the apical impulse seemed more diffuse, and the non-protein nitrogen was 79 mg. per cent.

He reentered the hospital in February, 1938. The temperature was 98.6, the pulse 120, respiration 32, and the blood pressure 240/150 mm. Hg. He was irrational and had Cheyne-Stokes respirations. There was no edema. Narrowing and obliteration of the vessels of the fundi with small hemorrhages were seen. The breath was not uremic. Dullness and sticky râles were present at the base of the left lung posteriorly. The heart was diffusely and greatly enlarged to the left, with heaving apex impulse, rapid rate, A_2 greater than P_2, hollow blowing apical systolic murmur and gallop rhythm. He had a convulsion lasting half an hour characterized by clonic spasm in the extremities and jaws, with loss of consciousness and incontinence of stool and urine. The hemoglobin was 65 per cent, red blood cells 3,140,000, white blood cells 14,800, and polymorphonuclears 87 per cent. The guaiac test on a stool was 2 +. The specific gravity of the urine was 1.008–1.011, albumin + to +++, glucose 0, and there were 2 to 4 red blood cells on occasional specimens but no casts. The serum phosphatase showed 5.4 Bodansky units per cent, inorganic phosphorus 6 mg. per cent, calcium 10.5 mg. per cent, non-protein nitrogen 110 mg. per cent, cholesterol 258 mg. per cent, protein 5.7 mg. per cent, albumin 3.9 mg. per cent, and globulin 1.8 mg. per cent. An X-ray revealed the outline of a horseshoe kidney containing large amounts of calcium, but when compared with the previous film there seemed to be no change in the extent or density of these calcium shadows.

The patient developed bronchopneumonia in both lungs with rapid respiration, became comatose and died.

Clinical Diagnoses: Healed hyperparathyroidism, calcification of horseshoe kidney, uremia, cardiac hypertrophy, secondary hypertension, and bronchopneumonia.

Abstract of Autopsy *

Permission could not be obtained to remove the calvarium. For purposes of completeness and brevity it may be said that the spleen, liver, gall bladder, pancreas, adrenals, bladder, prostate, seminal vesicles, right testis, alimentary tract, thyroid, thymus, superior pubic ramus, vertebrae and cortex of the left femur appeared normal in gross. The significant findings were as follows:

Heart: Weight 480 gm. The left ventricle measures 2 cm., the right 5 to 6 mm. in thickness. There is definite hypertrophy of the left ventricle. The valves are normal. The coronary arteries show slight atheroma without calcification. There is no gross evidence of fibrosis.

Lungs: The right lung weighs 700 gm. It is somewhat boggy and on section the parenchyma is quite moist with numerous, mottled, darker red firm areas in no constant relation to the

* No. 12711.

bronchioles. The left lung weighs 550 gm. and is essentially similar to the right. The lower lobe shows the more marked changes, being firmer and darker red.

Kidneys: There is present a horseshoe kidney which is removed with the aorta and is not weighed. The right portion measures 10.5 by 4 by 2 cm., the left, 10 by 4 by 2 cm. The isthmus bridging the vertebral column joins the lower poles and measures 3 by 2 by 5 cm. The pelvis on each side is dilated but shows no evidence of injection. No stones are present. The ureters are only slightly dilated, the right measuring 1 cm. in internal circumference, the left 13 mm. The capsule strips with moderate difficulty. The renal surface is extremely irregular and finely stippled with small, usually spherical white prominences which are surrounded by injected renal tissue. These small prominences measure 1 mm. in greatest diameter. Section of the kidney, however, particularly as the knife passes through the medulla, gives the impression of an extreme degree of calcification, a granular gritty sensation being transmitted. The kidney cortex is defined with difficulty and measures at the most 3 mm. The radial and glomerular structures are clearly seen. There is abundant deposition of calcium in the medulla. These grayish yellow, granular calcium deposits cause the pyramids to contrast sharply with the cortex. There are small cystic areas to be seen in the cortex. Dilated calices extend almost to the renal surface. A stain of a thin section (1 to 2 mm.) with silver nitrate in the dark, according to Kesten's technique [12] for the demonstration of calcium in gross material, and clearing the specimen by Spalteholtz' method, reveals by transmitted light a most striking calcification of the renal pyramids. Calcium determinations * disclose an average for three specimens of 2122 mg. of calcium per 100 gm. of wet kidney; the specimens are from different portions of the kidney and contain respectively 1715, 2691 and 1962 mg. of calcium per 100 gm. (average from 9 normal kidneys, (12 determinations) 9.3 mg. calcium per 100 gm.).

Testes: The left testis is small, approximately one-third the size of the right. Its tunica is not thickened and the tubules do not string out readily. The epididymis is small and firm.

Parathyroids: The specimen removed at operation in 1933 was

* The methods used by Donohue, Spingarn and Pappenheimer [13] were employed for all determinations of tissue calcium.

found on the right side, arising from and partially embedded in the posterior aspect of the lower part of the right lobe of the thyroid. It was entirely surrounded by an extremely thin capsule and weighed 3.5 gm. The gland was oval and very soft. It measured 2.5 by 1.6 by 1 cm. The freshly cut surface was smooth and fairly homogeneous, the gland appearing divided and one-half slightly more yellow than the other. A few tiny petechial hemorrhages were seen within this soft smooth tissue; no trabeculae could be made out and there were no gross areas of degeneration.

Three parathyroids are located at autopsy. These are the left upper, weighing 177 mg.; the left lower, 129 mg.; and the right upper, 93 mg.; a total for the three glands of 399 mg. They are definitely enlarged but not unusual in color or consistence.

Ribs: A fusiform swelling of the fifth rib on the left is noted in the anterior axillary line. On cross section this presents an unusually thick but symmetrical cortex and prominent trabeculae. A similar though larger swelling is found anteriorly on the right eighth rib. A short distance from the head of the second rib there arises a "collar button" shaped prominence approximately 1.5 cm. in length which, projecting into the right pleural cavity, produces a dimple-like depression of the lung over it. This mass is firm and hard and on section reveals no cystic areas.

Microscopic Examination

Sections of heart, aorta, lungs, liver, spleen, pancreas, adrenal, kidney, bladder, prostate, testes, stomach, intestine, colon, thyroid, parathyroids, thymus, femoral marrow, vertebra, second and third rib (right) with "collar button" projection, fifth rib (left), eighth rib (right), superior pubic ramus and cortex of the left femur were studied histologically.

It may be mentioned briefly that there is histological evidence of myocardial hypertrophy with a moderate diffuse fibrosis, moderate arteriosclerosis, lobular pneumonia, acute splenic tumor, atrophy of the left testis with absence of spermatogenesis, and a rather striking hyperplasia of the cells of Leydig in each testis. No calcium deposits are found in any of the viscera except in the horseshoe kidney.

Deserving of more consideration are certain vascular changes,

the renal lesions, the condition of the bones examined and the parathyroids.

Vascular Lesions: The arterioles of the pancreas, spleen and adrenals are markedly sclerotic, some having undergone necrosis. In addition there is extreme arterial and arteriolar sclerosis in the kidneys which may well in part explain the renal atrophy (Fig. 5). Arterioles, particularly in the adrenal and periadrenal tissues, are frequently converted into hyaline masses with no evidence of a lumen (Figs. 6 and 7). Occasionally the vessel wall is widened, the intima is markedly thickened and darker areas with large yellowish green "glassy" deposits comprise the greater portion of the intima. Small thrombi are encountered occasionally. Numerous medium sized arteries of the pancreas are the site of an obliterative process that has frequently progressed to complete occlusion of the lumen; occasionally there has occurred recanalization and central hemosiderin deposition. Elastic tissue stains reveal fragmentation or complete destruction of the elastic components of the arterial wall. Similar vascular lesions are seen in the spleen. The arteriolar lesions in the kidney are even more striking and involve branches of all sizes. The basophilic change in the intima is particularly striking and the vessels appear larger, as though the thickening intima, as it encroached upon the lumen, pushed outward also the media and surrounding renal tissues. Many of the afferent arterioles are converted into hyaline masses and hyalinization is frequently seen in the thickened intima of larger arteries. Several arteries contain thrombi and others show evidence of recanalization. In places in the area of scarring calcium is deposited in the walls of the arteries.

Kidneys: Various sections disclose marked and consistent changes. The glomeruli are in many instances normal. The capsules of Bowman are delicate, the spaces empty and the glomerular tufts well defined. There are, however, a great many completely hyalinized glomeruli, while others show thickening of Bowman's capsule, and partial glomerular hyalinization. Occasional glomerular capillaries either contain thrombi or have become necrotic. Many of the convoluted tubules are normal; others contain inspissated casts frequently calcified. There are irregularly defined areas of tubular dilatation. A few small areas of calcification are found in the interstitium of the cortex with slight dilatation of the

distal convoluted tubules. The most striking alteration, however, is seen in the region of the collecting tubules where there are large masses of calcium and complete destruction of tubules in many areas. Calcium extends as broad plaques through the pyramids replacing tubular and interstitial elements. Throughout the interstitial tissue of the cortex and medulla are numerous aggregates of lymphocytes.

Parathyroids: The tumor removed from the region of the right lower lobe of the thyroid is apparently well encapsulated by connective tissue, portions of which are hyalinized and contain hemosiderin. It is composed in greater part of extremely large cells grouped in small, oval or rounded nests of 5 to 20 cells enclosed by very slender connective tissue septums (Fig. 2). Strikingly apparent are stages of transition suggesting a relation between the four cell types. These are: (1) large cells, measuring 25 to 35 microns, oval and spherical, and frequently polyhedral in outline. The nuclei measure 15 to 25 microns, are usually spherical and centrally located but vary considerably in shape; they are frequently vesicular and contain a rich and irregularly arranged chromatin network. The nucleolus is prominent and eccentrically located, occasionally staining with eosin. The cytoplasm is composed of pinkish granular material, varying considerably in amount and frequently seen only at the cell periphery, leaving a clear halo about the nucleus; at other times the granules are more compact and the halo is absent. This type of cell is the most numerous. It is probably a neoplastic derivative of the chief cell or of the transition water clear cell. (2) Rarely, water clear cells are seen, though many of the cells considered to be derivatives of the chief cell have lost almost all cytoplasmic granularity and resemble closely the water clear cell; these are somewhat smaller than the chief cell, measuring 15 to 25 microns, the vesicular nuclei measuring 5 to 8 microns. (3) Nests of brilliant red polyhedral cells with compact granular cytoplasm and hyperchromatic nuclei are present. These cells measure 25 to 35 microns, the nuclei 10 microns. (4) In the midst of the tumor is a small island composed of smaller brilliant red cells with intensely hyperchromatic pyknotic nuclei; these cells measure 7 to 15 microns, their nuclei 5 to 7 microns. Occasionally various cell types form syncytia within the nests, at other times a single

cell appears to contain 2 to 3 nuclei. Mitoses are infrequently seen.

At the periphery of the tumor and lying just beneath the capsule is a rim of normal parathyroid tissue. Chief cells measuring 7 to 8 microns are predominant; their nuclei, usually vesicular, measure 5 microns. Numerous water clear elements and transitional forms are present. There are short columns of small, dark oxyphilic cells measuring 6 to 7 microns, with intensely hyperchromatic nuclei 3 to 4 microns in diameter.

The histology of the other three glands is essentially similar. They are vascular and hyperplastic, with no fat and only a moderate amount of stroma. The chief cell measuring 7 to 8 microns is predominant though many water clear and transitional forms are seen (Fig. 3). In several areas the chief cells are arranged in cords; more frequently they form closely packed groups. Some of the nuclei are hyperchromatic; others are vesicular. They measure 3 to 5 microns. The cytoplasm is finely granular, staining either blue or bluish pink. In many cells there is a perinuclear halo with transition to cells with completely water clear cytoplasm. The water clear cells are usually somewhat larger, measuring 8 to 12 microns with vesicular or hyperchromatic nuclei measuring 6 to 7 microns. Oxyphils with very finely granular cytoplasm are seen either isolated or in small groups. They are polyhedral in outline and measure 10 to 13 microns; their nuclei are hyperchromatic and measure 4 to 5 microns in diameter. In the right upper gland is a nest of approximately 50 similar cells. Occasionally these cells also show a perinuclear halo. In each gland are follicles, some of moderate size and each containing a homogeneous, eosinophilic colloid-like substance.

The glands are otherwise of interest in that at the periphery of both the left upper and left lower glands thymic tissue is in intimate relation with the capsules.

Ribs: The second and third (right), with projecting "collar button" nodule, appear normal. The cortex is not thickened and the trabeculae are slender. There is no osteoclastic activity or fibrosis of the marrow. A moderate amount of hematopoietic tissue is embedded in the marrow fat. The projecting mass is composed of osteoid tissue encapsulated by periosteum continuous with that of the second and third ribs.

Eighth (right) and fifth (left) ribs, through fusiform swellings show the cortices to be irregularly and considerably thickened. The trabeculae at the periphery are broadened, short and somewhat irregular, those in the center are slender and more normal in appearance. The marrow cavity consists largely of fat and there is no fibrosis.

Vertebrae: The osseous elements are normal. There is no fibrosis of the marrow and hematopoiesis is active.

Pelvis: Sections reveal some thickening of the cortex; the trabeculae at the periphery are broad and similar to those seen in the ribs. There are small areas of fibrosis in the marrow and a moderate number of osteoclasts with some evidence of osteoblastic and osteoclastic activity.

Discussion

There are three problems in this case that merit discussion. These are the mechanisms that resulted in the nephrocalcinosis, the etiology of the hypertension and the vascular alterations, and the nature of the parathyroid enlargement.

Donohue, Spingarn and Pappenheimer [13] have shown an increase in the calcium content of the residual renal tissue in rats rendered uremic by experimental reduction of kidney substance, but in our case extensive renal calcification occurred many months before nitrogen retention appeared. The only evidence of renal damage when the patient was first observed was the presence of slight albuminuria and a failure to concentrate the urine to a specific gravity greater than 1.012. This may have been due to the diuresis accompanying hyperparathyroidism. Suggested in the X-ray and strikingly apparent in the specimen is the concentration of calcium in the pyramids, whereas the cortical arches and columns of Bertini are relatively unaffected. Apparently the renal changes in this case are concerned primarily with the formation and inspissation of calcium casts in the collecting tubules. Lubarsch (Albright and Bloomberg [1]) describes calcium phosphate casts seen in hyperparathyroidism. Albright and Bloomberg suggest that the factors governing nephrocalcinosis are similar to those concerned with the precipitation of calcium phosphate ***in vitro***, ***i.e.*** the pH of the solvent and the concentration of calcium and phosphorus. The formation of stones has been recently discussed by Keyser.[14]

Although the mechanism of parathyroid hormone action is controversial, certain effects are known. Relative to nephrocalcinosis Chown, Lee and Teal [15] have experimentally shown extensive calcification of the kidneys following parathormone administration. Morgan and Samisch [16] and Olsen [17] have demonstrated chemically an increase of the calcium content of the kidney after injections of parathormone. We have produced in a rat by intraperitoneal injection an increase of renal calcium comparable to that found in our case. Clinically hyperparathyroidism is associated with an increased calcium and phosphorus excretion in the urine. Therapeutically and experimentally in man and in animals parathormone administrations increase the urinary output of calcium and phosphorus.

In the case reported here the pH of the urine was not known at the time the calcium deposition in the collecting tubules took place, nor is there a history of pyelitis or of renal calculi. There was evidence of a high urinary calcium and phosphorus excretion, as shown by balance studies, and this was probably increased by long continued calcium, viosterol and ultraviolet ray therapy. It seems fair to conclude that renal calcinosis in this case resulted from an increased concentration of calcium and phosphorus in the glomerular filtrate, brought about by excessive parathyroid activity. Calcification of necrotic renal parenchyma is present to some extent but the nephrocalcinosis found appears to differ only in the site of calcium deposition from nephrolithiasis associated with hyperparathyroidism. In the case discussed here the deposition of calcium in the urinary excretory passages has occurred at a point nearer to Bowman's space than the renal pelvis, namely in the collecting tubules.

The second problem is the interpretation of the vascular lesions. It may be assumed : (1) either that there was present a coincidental, essential or primary hypertension with generalized arteriolar sclerosis and necrosis responsible in part for the rapid development of renal insufficiency; or (2) that the generalized vascular lesions are secondary to the renal alterations. The patient was seen occasionally in the out patient department, but it was not until almost 4 years after the operation that symptoms of hypertension appeared; during this interval there are no records of the blood pressure. Near the end of this 4 year period definite hypertension

was evident. There followed rapid progression of the hypertensive cardiovascular renal disease.

It is not believed that so-called primary hypertension can be ruled out as a complication. The hypertension apparently did not appear until almost 4 years after extensive nephrocalcinosis had been demonstrated by X-ray; hence the generalized arteriolar sclerosis and necrosis may perhaps be considered as indicative of the malignant phase of a primary hypertension. The age of the patient and the rapid progress of the disease may also be considered in favor of primary hypertension.

Primary or essential hypertension is a definite clinical-pathological entity of obscure etiology. The case here reported is complicated by a preceding nephrocalcinosis that may have had some influence on the development of the vascular lesions. For this reason further discussion of a possible relationship seems pertinent.

Without the presence of a coincidental primary hypertension it must be assumed that the deposition of calcium resulted in atrophy of the renal parenchyma. Such atrophy has long been associated with hypertension but is not found to be the primary factor in the generalized arteriolar sclerosis and necrosis present in this case. Goldblatt [19] has produced in dogs diffuse arteriolar hyalinization and necrosis similar to that seen in this case and usually considered to be characteristic of the malignant phase of primary hypertension. He points out that, experimentally, at least two factors are necessary. These are: (1) elevation of blood pressure (mechanical factor); and (2) renal ischemia (humeral factor). Previously [18] he had shown the importance of renal ischemia induced by clamping the renal artery in the production of the hypertensive state whether one or both kidneys were rendered ischemic. It was by severely constricting the renal arteries that he produced diffuse arteriolar hyalinization and necrosis; such vascular lesions were not found in the kidney, presumably because the mechanical factor was not present.

The massive calcification of the renal pyramids may have resulted in renal atrophy and sclerosis of the afferent arterioles of certain of the glomeruli with resultant increased intrarenal vascular tension. Furthermore, the deposition of the calcium near branches of the renal artery may have compressed them and produced ischemia in certain areas of the renal parenchyma. This

latter occurrence might have given further impetus to the hypertension; this possibility, however, cannot be proved. It is hazardous to assume that the decreased blood supply of an atrophied kidney is an ischemia as the ratio of blood flow to the amount of surviving parenchyma must be known to decide this. These considerations are offered as a possible explanation of this case which must otherwise be classified as one of nephrocalcinosis complicated by an independent malignant essential hypertension.

The stimulus to enlargement of the parathyroid glands so regularly accompanying renal insufficiency is not known. The three parathyroids showed an enlargement of approximately 465 per cent by weight, resulting from hyperplasia of the epithelial components. Nothwithstanding this, the serum calcium was normal and the serum phosphorus only slightly elevated. From this it may be assumed either that the enlarged parathyroids effectively controlled the concentration of calcium and phosphorus in the blood, or that a high serum phosphorus is not the immediate stimulus to the hyperplasia.

The total weight of the three glands found at autopsy was 399 mg. Pappenheimer and Wilens [6] give 85 to 86 mg. as the mean weight of the three corresponding parathyroid glands in a series of miscellaneous non-nephritics over the age of 10 years. Gilmour and Martin's weights [20] are essentially in accord. The very small amount of thymus IV and thymus III [21] found associated with the left upper and the left lower parathyroid respectively in this case probably did not significantly add to their weights.

The histology of the glands corresponds to that described by Castleman and Mallory as characteristic of secondary hyperplasia. In addition there was another alteration not stressed by them, namely a progressive clearing of the cytoplasm of the chief cells.

The histology of the parathyroid tumor removed at operation differs greatly from that of the normal parathyroid and of the glands found at autopsy. The rim of normal appearing tissue at its periphery leaves little doubt that it represents a true neoplasia rather than hyperplasia. Castleman and Mallory's classification [22] of the pathology of the parathyroid gland would suggest that it belongs in the category of "single chief cell neoplasia." In both the tumor and the hyperplastic glands there is a strong suggestion of transition between cell types.

Our case may be interpreted as one of primary hyperparathyroidism associated with an adenoma of the right lower parathyroid which was removed after extensive calcification of the renal pyramids had occurred. After an interval of 4 years there developed hypertension presumably associated with the arteriolar sclerosis; this served at least in part to precipitate the uremic state in which the patient died. The enlargement of the remaining parathyroids is regarded as secondary to the renal insufficiency.

Summary

1. A case of hyperparathyroidism is reported associated with an adenoma of the right lower parathyroid, accompanied by calcification of a horseshoe kidney as disclosed by X-ray. Removal of the parathyroid tumor was followed by termination of the hyperparathyroidism. There developed subsequently hypertension, cardiac hypertrophy, renal insufficiency and death in uremia.

2. The important lesions disclosed at autopsy were extensive calcification of the renal pyramids of a horseshoe kidney, widespread arteriolar sclerosis and necrosis, arteriolar nephrosclerosis, and hyperplasia of the three remaining parathyroid glands.

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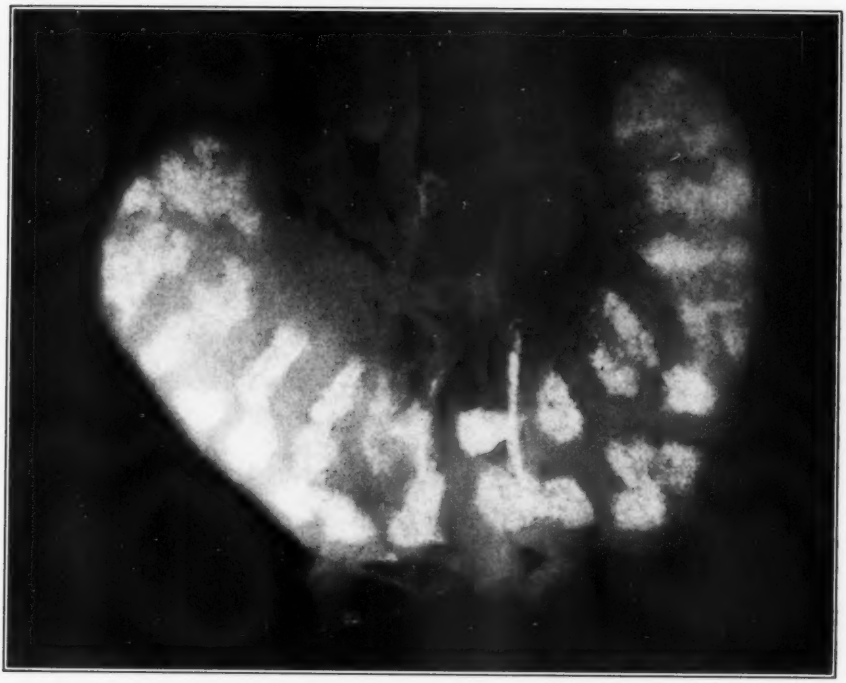
DESCRIPTION OF PLATES

PLATE 25

FIG. 1. Roentgenogram of horseshoe kidney removed at autopsy showing calcium deposits in pyramids and relative freedom of cortex and columns of Bertini from calcification. A calcified plaque is demonstrated in the aorta.

FIG. 2. Microphotograph of tumor of right lower parathyroid removed at operation showing large cells grouped in nests. Hematoxylin-eosin stain. × 700.

FIG. 3. Left lower parathyroid removed at operation, showing transitional forms between chief cells and cells resembling chief cells but with clear cytoplasm. Hematoxylin-eosin stain. × 700.



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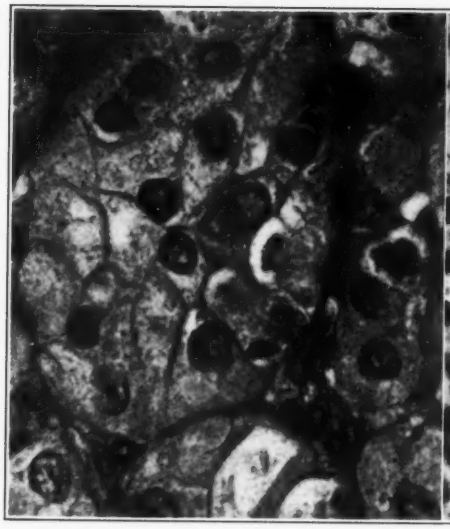

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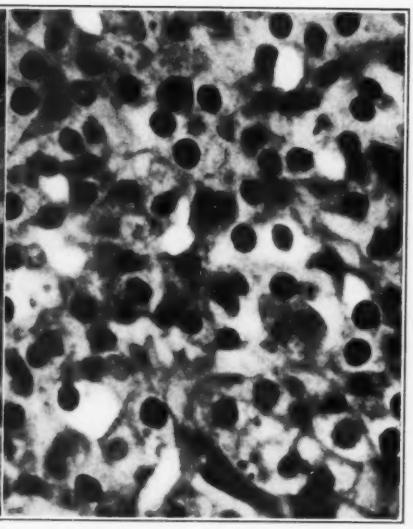

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Johnson

Primary Hyperparathyroidism

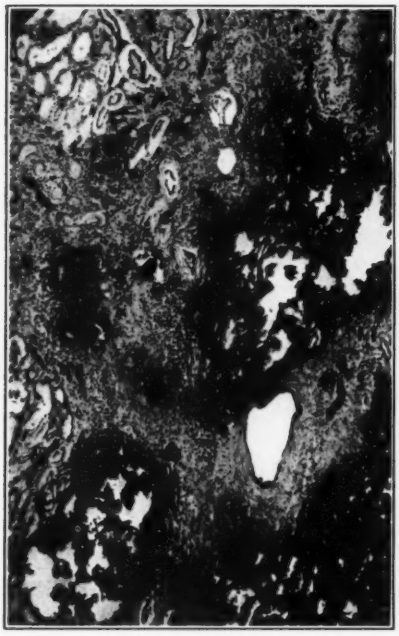
PLATE 26

FIG. 4. Massive deposits of calcium in pyramids of kidney and moderate dilatation of tubules. Hematoxylin-eosin stain. × 60.

FIG. 5. Advanced arteriolar nephrosclerosis, plugging of tubules by calcium and deposition of calcium in partially occluded arterioles. Hematoxylin-eosin stain. × 110.

FIG. 6. Extensive intimal thickening with hyalinization of a pancreatic arteriole. The lumen is almost completely occluded. Hematoxylin-eosin stain. × 110.

FIG. 7. Adrenal arteriole, extensive arteriolar necrosis with swelling and hyalinization of all elements of the wall and complete occlusion of the lumen. Hematoxylin-eosin stain. × 110.



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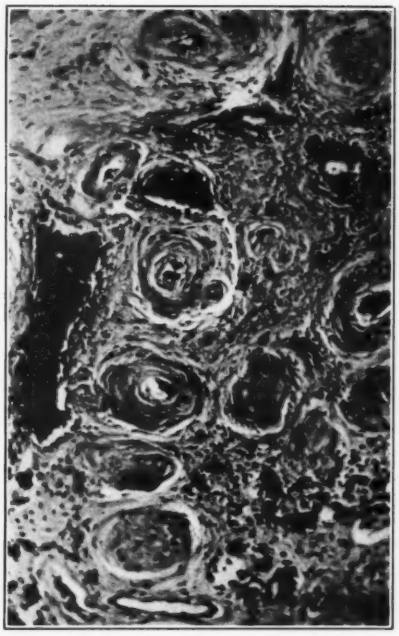

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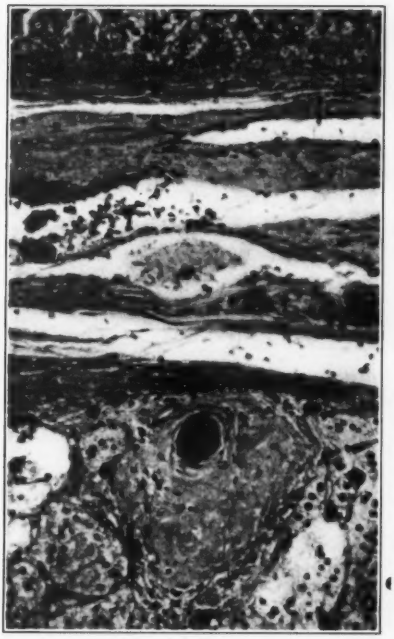

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Johnson

Primary Hyperparathyroidism

TULAREMIC SEPTICEMIA *

Report of a Case

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A case of tularemia with certain characteristics of an associated specific meningitis, which have not been emphasized heretofore, is reported. The primary lesion in the skin, which has been described only once in human beings, was available for histological study 19 days after the initial infection.

Report of Case

Clinical History: O. E., a white female, 52 years of age, was admitted to the hospital unable to speak and able to swallow only with great difficulty. Fifteen days before admission the patient was bitten on the left shoulder by a tick. Eight days later she complained of soreness in the left chest, associated with a slight cough, and at this time she felt that she had some fever. Two days later she began to have difficulty in swallowing and was unable to speak. The patient at no time complained of headache or vertigo. The past and family history were non-contributory to the present illness.

Physical Examination: The patient was well developed, obese, and semicomatose, unable to answer questions and mildly resistant to examination. The pupils reacted to light and accommodation normally and equally. Examination of the fundus revealed tortuosity and silver wire deformity of the vessels. The neck was rigid. Examination of the chest revealed no pathological findings on auscultation, percussion or palpation. The abdomen was slightly rigid and generally tender. All reflexes were hyperactive. A bilateral positive Kernig sign was elicited. A round ulcerous lesion with a scaly center, resembling herpes, was noted on the left shoulder. A lumbar puncture was performed and 45 cc. of cloudy yellow fluid under considerable pressure were removed.

Laboratory Examination: Urinalysis was essentially negative except for acetone and a few cells. Examination of the blood on the day of admission showed: red blood cells, 4,410,000; hemoglobin, 70 per cent; white blood cells, 11,100; polymorphonuclears, 77 per cent; and lymphocytes, 23 per cent.

An agglutination reaction 15 days after the tick bite was 1:160 for tularemia. Blood cultures remained sterile throughout hospitalization. Spinal fluid examination revealed on the day of admission, 1390 white blood cells with 94 per cent lymphocytes, sugar 13 mg., and chlorides 698 mg. Smears remained negative at all times for organisms. The total white blood cell count progressively fell to 110 with 91 per cent lymphocytes.

Course of Illness: Four more lumbar punctures were performed, each yield-

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ing cloudy fluid under pressure. Tache cérébrale was noted. Crepitant râles were present over the left lower chest posteriorly, associated with increased voice sounds.

The temperature on admission was 104.8° F. Fever continued throughout the patient's illness, the temperature never going below 102.8°, and it was recorded as 104.8° F. just before death, 4 days after admission to the hospital.

Pathological Findings

External examination reveals nothing of pathological interest except the left shoulder. On the posterior surface is a round, punched-out ulcer measuring 1.5 cm. in diameter and approximately 0.3 cm. in depth. The edges are somewhat ragged and there is a small amount of encrustation at the base and along the walls. It is surrounded by a purplish discolored area and numerous small vesicles which are situated intra-epithelially.

Lungs: The pleura is diffusely covered by fine fibrinous tags and shows numerous, firm, round raised nodules, varying in size from 0.3 to 1.5 cm. in diameter, which are made up of smaller nodules which have in places coalesced. The cut surface reveals diffusely distributed, gray, nodule-like foci in acinous arrangement with yellow necrotic centers. The structures around the hili are negative except for a few enlarged lymph nodes with coalescent areas of caseation.

Spleen: The spleen weighs 280 gm. and measures 15 by 12 by 2 cm. The capsule is wrinkled and the organ is soft. The cut surface is dark red and the follicles and trabeculae are barely visible. There are several, large, (0.2 to 0.3 cm.) firm yellow nodules lying within the splenic pulp, which can be scraped away on the knife edge.

Liver: The liver weighs 1700 gm. and measures 30 by 23 by 9 cm. There are pin-head-sized and smaller tubercle-like foci diffusely scattered over the cut surface, which in places are definitely situated between the lobules.

Head: The scalp and calvarium are negative. The meninges present a milky opacity and show innumerable, pin-head-sized tubercle-like foci diffusely scattered throughout the subarachnoid space over all parts of the brain. There is a slight amount of turbid, yellowish gelatinous exudate in the subarachnoid space, rather diffusely distributed but most prominent at the base of the brain.

Microscopic Examination

Characteristic tularemic nodular areas of necrosis are found in the spleen, liver, lungs, lymph nodes, skin and meninges.

Lungs: Sections through various parts of the lungs show two types of lesions.

(1) Numerous nodules with epithelioid cells and central caseation are present in the interstitial tissue. Wherever they encroach upon a vessel they produce complete necrosis of the wall. Intimal proliferation of small vessels in the vicinity of these foci is occasionally encountered.

(2) Areas of caseous bronchopneumonia, some of which are acinous in distribution, are found in all sections. The exudate consists mainly of large mononuclear cells, plasma cells and some fibrin, the latter often clumped. In places this exudate is necrotic. In other areas where the alveolar wall is still intact the vessels are patent but often engorged, and minute hemorrhages frequently occur. The alveolar walls in other areas are necrotic.

In addition to these specific changes there are widely scattered areas of non-specific bronchopneumonia and aspiration pneumonia with recognizable foreign material in the alveolar lumens.

Brain: The meninges are edematous. The subarachnoid space shows an exudate composed of polymorphonuclear cells and a variety of round cells, among which large mononuclear cells predominate. These are actively phagocytic, containing small mononuclear cells, nuclei and nuclear fragments, and are frequently foamy in appearance. In some areas the large mononuclear cells accumulate and form rather discrete nodular foci. Fibrinous exudate is seen within and around these foci. Polymorphonuclear cells are rather scanty and epithelioid and giant cells are absent. The foci are irregularly distributed but the majority are found to be independent of vessels, even in the early stage. However, they encroach occasionally upon a large artery producing necrosis of all coats of the wall with proliferation and infiltration of the intima. Thrombi are not encountered. Non-specific round cell infiltration follows the perivascular lymph spaces of some of the smaller vessels into the outer layer of the cortex. This infiltration, however, does not form specific nodules.

Many of the meningeal foci invade the cortical substance, pro-

gressing into the uppermost zone and infiltrating the outer layer of ganglion cells. A Nissl stain reveals that the ganglion cells resist the destructive process for a long time. Although axis cylinders and myelin sheaths have been destroyed and the ganglion cells are compressed within such areas, they reveal normal distribution of tigroid substance and normal nuclei. Their processes seem, however, often to be unduly swollen.

Sections taken from the pons, brain stem, Ammon's horn and various portions of the cortex appear to be normal.

Skin: A section from the area of the tick bite shows a deep ulcer, the base of which is within the level of the corium although close to the subcutaneous fat tissue. Its superficial layer is composed of débris containing numerous bacteria, mostly staphylococci, either isolated or in clumps. Beneath this is a layer of round cell infiltration consisting mainly of polymorphonuclear cells. The vessels beneath the ulcer are considerably engorged and there are numerous areas of hemorrhage scattered throughout the corium.

Another section taken from an area close to the tick bite shows the formation of a vesicle. The epidermis covering an area of dense infiltration is somewhat thickened. There is slight acanthosis, but most of the thickening seems to be due to a degenerative process in the prickle cell layer. The stratum corneum is lifted and forms the top of the vesicle; the base is level with the stratum lucidum.

A few, apparently early specific lesions are found just beneath the epithelial surface. Discrete areas of necrotic connective tissue are infiltrated, particularly at the periphery, with round cells, mostly of the lymphocytic type, and with a few polymorphonuclears. The infiltrating cells undergo rapid necrosis and a dense aggregation of nuclear fragments is seen between the necrotic masses of connective tissue. These areas of nodular necrosis border apparently normal connective tissue. There is no peripheral zone of monocytic or epithelioid cells. At one point this focus encroaches upon a small vessel, the wall of which is completely necrotic. There is some hemorrhage into the necrotic area.

For some distance from the base of the ulcer, separated from the surface by intact connective tissue, a few nodular areas of necrosis are seen resembling very closely the nodules found in

other organs. At the periphery of these necrotic areas large mononuclear and epithelioid-like cells are present together with a rather dense infiltration of round cells and a few polymorphonuclears. One of these nodules is situated at the root of a hair follicle destroying the squamous epithelial cell layer. It is associated with an accumulation of polymorphonuclear cells within the epithelial lining of the follicle resulting in necrosis of the hair shaft itself. The vessels of the papillae between the nodule and the epidermis are considerably engorged. Rather dense infiltration with lymphocytes is seen in perivascular and perifollicular spaces and around the sweat glands throughout the section. Some arterioles leading to specific foci show complete necrosis of the media, but the adventitia and intima are well preserved. The walls of other vessels are completely invaded by granulation tissue with destruction of the intima and early fibrinous thrombosis.

Comment

Both in gross and microscopically, the case presents all the characteristics of tularemia. However, certain observations warrant a discussion.

1. *The Meningitis:* Tularemic meningitis has been reported three times (Bryant and Hirsch,[1] Pund and Hatcher,[2] and Haizlip and O'Neil[3]). It has been stressed that in gross the lesions resemble tuberculous meningitis, except for the fact that in tularemia the base of the brain is involved to a lesser degree than the cortex and the lateral aspects of the hemispheres. Histologically, tuberculous and tularemic meningitis are almost identical. In each the nodular granulation tissue tends toward rapid necrosis, giant cells are scanty, and epithelioid cells poorly differentiated. There is a tendency in each to diffuse spread of a subarachnoid exudate which is predominantly of a mononuclear cell type and not specific in nature.

The distribution of nodules in our case of tularemia, however, differs strikingly from that seen in tuberculosis. While the nodules in the latter condition have a distinct tendency to follow the vessels, particularly into the depths of the sulci, there is an irregular distribution in our case independent of the course of the vessels with the site of the nodules preferably at the vertex of the gyri.

This difference is likewise noticeable in histological sections.

In tuberculous meningitis the lesion usually begins in the adventitia of the vessels and it is characteristic to find the epithelioid tubercles or foci of caseation in later stages predominantly in perivascular arrangement. Involvement of the media and intima is common, frequently resulting in occlusion of arteries by specific or non-specific endarteritis. Although the tuberculous process may occasionally encroach directly upon the brain tissue, there is, in general, little tendency to destructive invasion through the glial membrane.

In our case of tularemic meningitis the relations are almost reversed. The lesions begin some distance from the vessels where large mononuclear cells accumulate and form discrete nodular foci. The fully developed nodules of caseation in later stages seem almost to avoid the vessels, most of which are intact. The caseating granulation tissue only occasionally encroaches upon the wall of a vessel. The tularemic nodules, furthermore, show a distinct tendency frequently to invade the adjacent cortical tissue. In their description of tularemic meningitis, Bryant and Hirsch mention that the lesions were distributed chiefly, although not invariably, along the vessels. With the exception of this report we could not find specific statements in the descriptions of other cases of tularemic meningitis with reference to the relation of nodules to vessels. Their irregular distribution and their independence of vessels in our case are most striking and if this tendency is as salient as we noticed it to be, it may be of value in regard to the differential diagnosis from tuberculous meningitis.

2. *The Primary Lesion:* The primary skin lesion 15 days after infection has been described by Goodpasture and House.[4] Woolley [5] briefly reports the histological changes in primary skin lesions of experimental tularemia in laboratory animals. Goodpasture emphasizes the fact that the necrosis extends into the surrounding tissue particularly about the arteries which, however, are left relatively unimpaired.

In our case the intradermal spread occurred in a rather irregular fashion within the lymph channels independent of vessels. The cellular infiltration, which in areas showed a tendency to necrosis, was found in periglandular, perivascular and perineural lymph spaces. Nodules of mononuclear cells with caseation were also found isolated, in no relation to any one of the above men-

tioned structures of the skin. They encroached, however, not infrequently upon smaller vessels resulting in necrosis of a segment or the entire circumference of their walls with subsequent subintimal infiltration and proliferation.

The primary ulcerating lesion spread into the surrounding skin within a distance of several centimeters, causing numerous small blisters. The latter histologically resemble impetigo. The vesicles are situated within the epidermis proper, the horny layer lifted to form its roof. Hair follicles are involved in a suppurative process. It seems reasonable to assume that this vesicular dermatitis surrounding the primary lesion is due to cutaneous autoinoculation. It must, however, be emphasized that specific nodules were found independent of hair follicles. Our findings do not permit a definite conclusion with reference to the mechanism of intradermal or epidermal spread. It is most probable, however, that it follows the lymph channels.

3. *Tularemic Septicemia:* The involvement of rather numerous and engorged vessels in the specific inflammatory process of the skin would explain the mechanism of further hematogenous spread had it not been that Goodpasture's findings shed doubt upon this interpretation. The intracutaneous vessels in his case are described as relatively unimpaired.

It is of interest to note that most cases of tularemic septicemia lack the embolic type of metastases in the organs of the greater circulatory system. The case of Permar and Maclachlan [6] possibly represents an exception in that an anemic infarction occurred in the spleen which was believed to be due to thrombosis of a pulmonary vein. It is, however, uncertain whether this infarction was specific in nature or not. Metastases most frequently are confined to the lungs, lymph nodes, and organs with reticuloendothelial cells (mainly liver and spleen). Foci in other organs are rare exceptions (Pund and Hatcher [2]). Lymph nodes are often preferably involved. This distribution indicates that no special focus of development, such as a thrombophlebitis, is interposed between the focus of entrance and the spread of infection. In this respect tularemic septicemia differs from tuberculous septicemia (miliary tuberculosis) in which the focus of development can be detected in the great majority of cases as caseous thrombophlebitis of pulmonary veins or caseation of the thoracic duct.

Tularemic septicemia, furthermore, is distinguished from miliary tuberculosis in that it shows a tendency to become localized in lymph nodes, particularly those draining organs with tularemic foci of infection. It is characteristic to find caseation in gross in the hilar lymph nodes of the lung and liver if tularemic foci are localized in these organs.

4. *Involvement of Vessels:* It has been stated that endothelial and subendothelial proliferation frequently occur, resulting in narrowing of the arterial lumens, thereby causing the caseating necrosis of the tissue (Permar and Weil,[7] and Gundry and Warner [8]).

Although vascular changes with narrowing of the lumen were observed in our case, they were scanty, scattered and mostly confined to the lesions in the lung. The process of caseation, however, appeared to be independent of the vascular lesions. Extensive areas of necrosis were found in the lung in which the alveolar walls contained patent capillaries engorged with blood. In fact, hemorrhages into such foci from capillaries supplying these areas were fairly frequent. This proves that necrosis occurs while the blood supply is still intact. We conclude that the coagulation necrosis in tularemia does not depend on vascular changes and is not ischemic in origin.

Summary

1. A case of tularemic septicemia is presented with special reference to the primary skin lesion and the meningeal involvement.

2. The difference in distribution of lesions in tularemia and miliary tuberculosis is discussed and the following conclusions reached: (1) Tularemic meningitis is characterized by relative independence of nodular foci of the course of vessels. (2) Lymph nodes draining organs involved in tularemia show frequent caseation. (3) The distribution of nodular foci of infection in tularemic septicemia indicates that no intermediate focus of development is interposed between the point of entrance and the metastasis.

3. The process of caseation is not due to lack of blood supply since areas of caseation containing patent and engorged capillaries are demonstrated.

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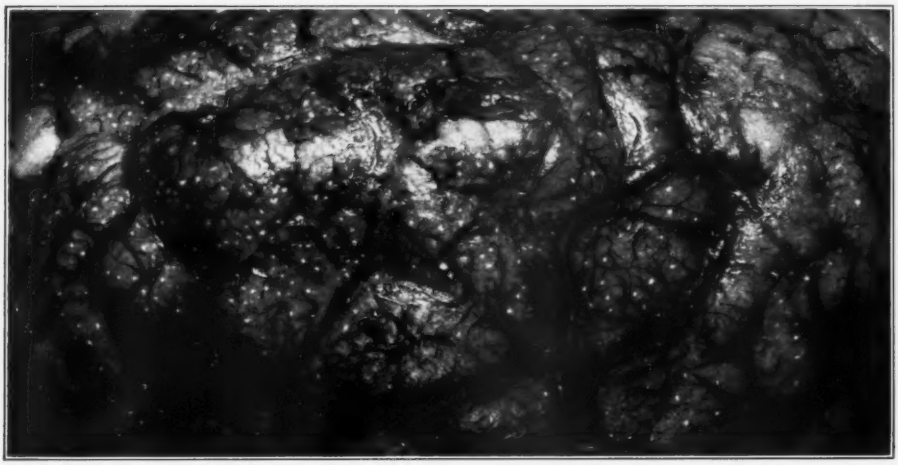
DESCRIPTION OF PLATE

PLATE 27

FIG. 1. Lateral view of brain. The tularemic nodules are rather large and appear relatively independent of the course of the vessels.

FIG. 2. Section of meninges showing a tularemic nodule fully developed and independent of the blood vessels. Even the blood vessel close to the large nodule shows a sharp line of demarcation.

FIG. 3. Blood vessel immediately beneath the primary lesion of the skin where the granulation tissue destroyed a segment of the wall, resulting in rupture of the endothelium.



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Kimmelstiel and Caldwell

Tularemic Septicemia

CONGENITAL RHABDOMYOMA OF THE HEART *

Report of a Case

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Von Recklinghausen [1] in 1862 was the first to report a case of congenital rhabdomyoma of the heart. Since then reports of 49 other cases have appeared in the medical literature. In America, cases have been recorded by Knox and Schorer (1906),[2] Wolbach (1907),[3] Farber (1931),[4] and Ill and Gray (1934).[5] The case here reported makes the 5th in American and the 51st in the general medical literature.

Report of Case

Clinical History: The mother of the infant to be discussed was a white woman, 24 years of age. The past history was irrelevant. The Wassermann reaction was negative. She had a normal spontaneous delivery of a child, now living and well, on May 9, 1934. A spontaneous abortion at 3 months occurred in August, 1934.

Later, the birth of a male infant, weighing 4215 gm., occurred on April 29, 1937, on the obstetrical service of Bellevue Hospital. At birth the child was cyanotic. Respiration was artificially established 3 minutes after birth, but the cyanosis continued. Normal temperature could be maintained only through the application of external heat. Physical examination of the baby was negative except for cyanosis and a short systolic murmur which was heard over the pulmonary cardiac area. The baby lived only 3 hours.

Postmortem Examination

The body is that of a well developed white male infant weighing 4215 gm. and measuring 53.3 cm. in length. The skin appears normal except for a moderate amount of postmortem lividity in the dependent portions of the trunk.

With the exception of the heart and brain, other organs show nothing of importance.

Heart: The heart is enlarged to twice its normal size and weighs 49 gm. The epicardium appears normal. On the left border of the

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heart, close to the apex, there is a raised nodule 1 cm. in diameter, which on section is seen to be composed of a circumscribed area of soft brown tissue arranged in whorls.

The pulmonary artery is dilated and measures 1 cm. in diameter at its base. The ductus arteriosus is dilated also, measuring 8 mm. in diameter, but the ascending aorta measures only 5 mm. It would appear that the main flow of blood was through the ductus arteriosus rather than through the ascending aorta. The descending aorta appears normal.

On sectioning the heart the right auricle and tricuspid valves appear to be normal. A rudimentary interauricular septum imperfectly separates a rudimentary left auricle, measuring 1.5 cm. in thickness, from the right auricle. The foramen ovale cannot be identified. The left auricle through the atretic mitral valves communicates with a small cavity 1.5 cm. in diameter representing the left ventricle. The aortic valves are bicuspid. The right ventricle is lined with glistening endocardium. The papillary muscles are hypertrophied and the base of one of the muscles within the right ventricle has been invaded by a tumor measuring 1.5 cm. in diameter. The left ventricle is lined with glistening gray endocardium 2 mm. in thickness. Adjoining the endocardial lining is a layer of dense gray tissue 2 mm. thick. The wall of the right ventricle is 1 cm. thick. The wall of the myocardium representing the interventricular septum measures 3 cm. and separates the rudimentary left ventricle from the large right ventricle.

On section of the interventricular septum two distinct tumor masses are seen. One measures 2.5 by 1.5 cm. It is well encapsulated, gray in color, and extends into the base of the papillary muscle as mentioned above. The cut surface of the tumor presents a grayish, granular dimpled appearance with faint, peripheral radial striations adjacent to the capsule. The capsule is 1 to 2 mm. thick and is smooth, gray and glistening.

The second tumor, 2 by 2.5 cm., is adjacent to the one described above and extends through the remainder of the interventricular septum and the anterior wall of the right ventricle. The tumor is not encapsulated but is well defined from the surrounding myocardium. It is composed of smooth, firm, glistening gray tissue somewhat fibrous in appearance. Scattered through the myocardium at the apex of the heart are a number of well circum-

scribed, light brownish islands of tissue of the same consistence as the myocardium.

Brain: On opening the cranium a moderate amount of subarachnoid edema is found and the cerebral vessels appear moderately congested. On section of the brain the subependymal tissue of the lateral ventricles is markedly thickened. On the floor of the left lateral ventricle extending posteriorly through the left occipital lobe is a tumor 6 by 2 cm. composed of well circumscribed reddish brown tissue. The tumor is firmer in consistence than the surrounding brain tissue. There is a moderate degree of internal hydrocephalus.

Microscopic Examination

Heart: A hematoxylin and eosin stain of tissue taken from the interventricular septum shows a tumor with a thin connective tissue capsule which varies in thickness, and in a few areas the tumor lies directly superimposed on myocardial tissue. A number of fair sized blood vessels with thin walls are seen within the capsule. Short connective tissue septums dip into the tumor, carrying small and medium sized blood vessels. A few small or medium sized arteries are also found within the tumor.

The myocardium immediately overlying the capsule is composed of delicate and elongated muscle fibers with prominent nuclei and cross striations and separated by slender connective tissue fibers containing a few fresh red blood cells and leukocytes.

The tumor is characterized by many, usually rounded vacuolated spaces which vary in size (Figs. 1 and 3). The tissue between them is composed of large polygonal branching cells with a finely granular and eosinophilic cytoplasm (Fig. 3). A fine fibrillar network is associated with these cells. Three or four cytoplasmic processes spring from the surface of each cell body and many of the cells contain round, deeply staining nuclei. In some instances the cells appear to lie within the vacuoles, the cytoplasmic processes spreading out to subdivide the space (Fig 2).

With high magnification the vacuolated spaces are seen to be lined by a thin rim of amorphous tissue. Often the lining of these spaces appears to be derived partially from the cytoplasmic process of an adjacent large granular cell (Fig. 3). The spaces appear to be extracellular and the large polygonal branching cells

lie between them. The majority of the spaces are clear but a few, however, contain several small round eosinophilic cells with bilobed dark nuclei resembling polymorphonuclear leukocytes (Fig. 1).

The cytoplasm of the cells contains many fine eosinophilic granules arranged to produce cross striations (Figs. 1 and 2). Under oil immersion the cross striations become even more evident and are seen to extend from the main body of the cell into the branching processes, which number two to six per cell. Most of the cells contain a single, large round nucleus with prominent nucleoli and chromatin granules. A few of the cells contain two nuclei.

Sections of the apparently normal myocardium disclose small foci of tumor tissue similar to that described above. A section of the second tumor mass shows tissue resembling a fibromyxoma, representing, probably, degenerative changes.

Brain: On microscopic examination the tumor in the occipital lobe is found to be a spongioblastoma with unipolar, bipolar and multipolar cells. Between the spongioblasts are large numbers of small, oval and round nuclei which cannot be identified. Every portion of the brain examined shows some deviation from normal. The main changes are noted in the occipital and frontal poles on both sides. Here the cortical architecture is distorted. Many abnormal nerve cells are present as well as enormous astrocytes and numerous undifferentiated cells. No ependymal cells are found lining the ventricles, but replacing them is a dense layer of glia fibers. No myelin formation is noted in any of the sections.

Other Organs: The lungs are congested and show focal hemorrhages with foci of aspirated amniotic fluid. Icterus is present in the liver. The kidneys are congested. The spleen shows acute splenitis.

Pathological Diagnoses: Primary congenital rhabdomyoma of the heart with multiple tumors; congenital malformation of the heart with atrial septal defect, hypoplasia of the aorta, mitral stenosis, dilatation of the pulmonary artery and ductus arteriosus, bicuspid aortic valve, and rudimentary left auricle and left ventricle; and tuberous sclerosis of the brain with cerebral tumor.

Discussion

In Table I are summarized the cases reported to date of congenital rhabdomyoma of the heart.

Of the 51 cases the tumor was classified as single in 8, multiple in 36, and diffuse in 3. Two cases showed two nodules, 1 case showed no tumor in gross, and 1 other case is unquestionably a rhabdomyoma from the photographs. Tuberous sclerosis of the brain was found associated with the tumor of the heart in 29 cases. In 4 cases tuberous sclerosis was not found and in 18 cases no mention of this lesion is made. Other congenital lesions were found also in association with the rhabdomyoma of the heart. Thus, in 24 cases tumors or cysts of the kidneys were reported. Cutaneous tumors were seen in 3, an enlarged liver or spleen in 3, and harelip or cleft palate in 1 case. Thirty-one cases had some associated lesion in organs other than the brain or heart. The age distribution of this tumor was found to be as follows: newborn, 10; under 1 year, 15; 1 to 3 years, 8; 3 to 15 years, 11; and over 15 years, 5. The age was not given in 2 cases.

As evidence of the congenital nature of rhabdomyoma of the heart, its occurrence in the newborn and its association with congenital anomalies have been noted. Cleft palate, harelip, cystic kidneys, tuberous sclerosis, multiple gliomas of the brain, hypernephroma, sebaceous gland adenomas, embryonic rests in the kidney, and embryonic malformation of the pancreas are among the more important congenital anomalies found associated with rhabdomyoma of the heart. Neuropathologists have noted that congenital anomalies are often associated with tuberous sclerosis of the brain.

Steinbiss [6] believes that the congenital rhabdomyomas of the heart cannot be regarded as true tumors. They do not show any evidence of proliferative activity, and degenerative changes such as fibrosis and calcification have been noted in older lesions. In the case here reported, a small portion of the tumor showed fibrotic degenerative changes. Malignant transformation of the tumor has not been recorded and probably does not occur. Farber [4] gives an excellent review of this controversial question. He believes that the evidence is against a neoplastic nature of the tumor. Rehder,[7] and Schmincke,[8] as well as Steinbiss believe that

TABLE I

Data on Cases of Congenital Rhabdomyoma of the Heart Reported in the Literature to Date

(Enlarged after Farber)

Number	Author	Year	Age	Sex	Histological findings		
					Heart	Brain	Other organs
1	Von Recklinghausen [1]	1862	Newborn	–	Multiple nodules in both ventricular walls	Tuberous sclerosis	Cutaneous tumors
2	Virchow [21]	1864	Newborn	–	Multiple nodules in both ventricular walls	——	Hepatomegaly, multiple skin tumors
3	Hlava [22]	1887	14 days	–	Single tumor in left ventricle	Not examined	——
4	Kolisko [18]	1887	2 mos.	–	Multiple small nodules	——	——
5	Cesaris-Demel [20]	1895	3 yrs.	–	Multiple tumors in both ventricles and septum	Tuberous sclerosis	Small nodular renal tumors, embryonic renal tissue without glomeruli
6	Seiffert [17]	1900	20 mos.	M	Multiple tumors in apex, myocardium and septum	——	Cystic kidney
7		1900	7 mos.	–	Multiple small myocardial tumors	——	——
8	Rothe [23]	1901	–	–	Multiple tumors	Tuberous sclerosis	Multiple breast tumors
9	Ponfick [19]	1901	7 mos.	M	Multiple nodules in both ventricles	Tuberous sclerosis	——
10		1901	3 yrs.	F	Multiple nodules in both ventricles	Tuberous sclerosis	——
11	Bonome [16]	1902	1½ yrs.	–	Multiple tumors	Tuberous sclerosis	——

12	Riedmatten [24]	1904	1½ yrs.	–	Multiple tumors	Tuberous sclerosis	——
13	Knox and Schorer [2]	1906	7 mos.	–	Multiple tumors, one large pedunculated tumor in left ventricle	——	——
14	Wolbach [8]	1907	10 mos.	F	Single tumor in right ventricle	Negative	Neuroglioma of spinal meninges
15	Abricossoff [13]	1909	3¼ yrs.	–	Multiple tumors in both ventricular walls	Tuberous sclerosis	——
16	Ehrnrooth [25]	1911	7 mos.	–	Single tumor in left ventricle	Grossly negative	Negative
17	Bundschuh [26]	1912	2 yrs.	F	Multiple nodules	Tuberous sclerosis	Tumors of kidneys, glioma of dura, adenoma sebaceum
18	Jonas [27]	1912	6 mos.	M	Multiple nodules in both ventricular walls	Tuberous sclerosis	Congenital malformation of kidney, hare-lip, cleft palate
19	Kawamura [9]	1913	4 yrs.	F	Multiple nodules in both ventricles	——	Renal tumors, congenital anomalies in pancreas, esophagus and rectum
20	Schulgin [10]	1913	6 days	–	Multiple tumors in both ventricles	Tuberous sclerosis	Kidney tumors
21			6 yrs.	–	Multiple tumors in both ventricles	Tuberous sclerosis	Kidney tumors
22	Rehder [7]	1914	Newborn	–	Multiple nodules	——	Grossly negative
23	Mönckeberg [11]	1914	14 mos.	–	Multiple nodules	Tuberous sclerosis?	Absent right kidney and ureter
24	Ribbert [28]	1915	1 yr.	–	Multiple nodules	Tuberous sclerosis	Cysts of kidneys
25	Hisinger-Jägerskiöld [29]	1916	7½ mos.	–	Single nodule at apex	——	Tumors of kidneys
26	Amersbach and Handorn [15]	1921	7 days	M	Single nodule	Negative	Negative

TABLE I (*Continued*)

Number	Author	Year	Age	Sex	Histological findings		
					Heart	Brain	Other organs
27	Kaufmann [20]	1922	3 yrs.	–	Multiple nodules	Tuberous sclerosis	——
28		1922	7 yrs.	–	Multiple nodules	Tuberous sclerosis	Kidney tumors
29	Mittasch [21]	1922	4 mos.	–	Multiple nodules	Tuberous sclerosis	Kidney tumors
30		1922	14 yrs.	M	Multiple nodules	Tuberous sclerosis	Kidney tumors
31		1922	31 yrs.	M	Multiple nodules	Tuberous sclerosis	Kidney tumors, angiomyolipoma of liver
32	Schmincke [8]	1922	Newborn	–	Diffuse involvement of myocardium	——	Congenital tumor of lungs
33	Steinbiss [6]	1923	5 yrs.	M	Multiple nodules in both ventricles	Tuberous sclerosis	Fibroepithelioma of skin, kidney tumors?
34		1923	8 yrs.	M	Multiple nodules in both ventricles	Tuberous sclerosis	Kidney tumors
35		1923	10 yrs.	M	Multiple nodules in both ventricles	Tuberous sclerosis	Kidney tumors, adenoma sebaceum
36		1923	16 yrs.	M	Nodule in septum and cicatrix	Tuberous sclerosis	Kidney tumors and cysts
37		1923	21 yrs.	F	Two small nodules in right auricle	Tuberous sclerosis	Kidney tumors
38		1923	35 yrs.	–	Single nodule at apex of left ventricle	Tuberous sclerosis	Kidney tumors
39	Omodei-Zorini [22]	1923	2½ yrs.	–	Single tumor in right ventricle	——	——

40	Uehlinger [13]	1925	20 yrs.	M	Multiple nodules in left myocardium	Not mentioned	——
41	Berger and Vallée [14]	1930	2 yrs.	–	Multiple nodules	Not examined	Cysts of kidneys
42	Farber [4]	1931	6 mos.	F	Multiple nodules in both ventricles	Tuberous sclerosis	Cysts of kidneys
43	Reitano and Nucciotti [33]	1933	1 day	–	Multiple, one large nodule almost replacing the entire heart	Not mentioned	——
44	Ill and Gray [5]	1934	48 hrs.	M	Multiple nodules	Not examined	Enlarged liver and spleen
45	Mitani [23]	1934	Newborn	M	Single tumor in interventricular septum and muscle of ventricles and auricles	Tuberous sclerosis	Cysts of kidney
46	Wegman and Egbert [34]	1935	10 mos.	F	Multiple nodules	Negative	Cysts of kidneys
47	Heuper [35]	1935	7 mos.	M	None grossly, microscopic foci of tumor tissue	Tuberous sclerosis	Cysts of kidneys, multiple spongioblastomas of basal ganglia, enlarged liver
48	Pauli [37]	1936	4 mos.	M	Diffuse tumor	Not mentioned	Enlarged liver, hydrocele
49		1936	6½ mos.	M	Diffuse tumor	Not mentioned	Not mentioned
50	Tamura [36]	1936	–	–	From photographs is unquestionably a case of rhabdomyoma	——	——
51	Labate	1939	3 hrs.	M	Multiple tumors in right ventricle and interventricular septum	Tuberous sclerosis with spongioblastoma	Congenital anomaly of heart

the tumor is a malformation and not a true tumor. The form of the embryonal cells composing the tumor is retained but the size is greatly increased. There is retardation of development but hypertrophy of the individual elements.

On the other hand, Wolbach [3] with the aid of the phosphotungstic acid hematoxylin stain demonstrated beginning muscle fibril formation and so considered the rhabdomyoma a true neoplasm. He demonstrated that the walls of the vacuolated spaces contain striated fibrils, analogous to the fibrils of normal heart muscle. These striations were observed in studying our case. Many observers have recalled the analogy between the tumor cells and embryonic heart muscle.

Knox and Schorer,[2] Kawamura,[9] Schulgin,[10] Mönckeberg,[11] Abricossoff,[12] Uehlinger,[13] and Berger and Vallée [14] believed that the cells of the tumor arise from Purkinje fibers.

Steinbiss claimed that the tumors were found often where the conduction system could not be demonstrated. Amersbach and Handorn [15] also believed that no connection existed between the tumor and the conduction system. Bonome [16] believed that rhabdomyomas develop from embryonic muscle fibers which become isolated through a connective tissue overgrowth replacing adjacent degenerated muscle fibers.

Wolbach, with Mallory's aniline blue connective tissue stain demonstrated that the sarcous elements stain red and the delicate striations between the sarcous elements (membrane of Krause) blue. In tumor tissue stained in this way the delicate fibrils connecting the fuchsin stained bodies are blue. With the phosphotungstic acid hematoxylin stain they are a reddish brown. These facts justify the belief that the granules of the tumor cells are primitive sarcous elements. The orderly arranged dots, most prominent in the tumor cells and the cell processes, are primitive sarcous elements. The fibrillary material taking the blue of the connective tissue stain is to be regarded as an element similar to that of Krause's membrane in normal muscle.

Von Recklinghausen,[1] Seiffert,[17] Kolisko,[18] Ponfick,[19] and Bonome [16] have noted the similarity of the tumor cells to embryonic heart muscle cells.

Von Recklinghausen considered the vacuolated spaces to be lymph or blood spaces, or muscle tubes of pathological origin.

Cesaris-Demel [20] was the first to call attention to the many processed cells lying within the spaces and called them "spider cells." He regarded the spaces as intercellular and similar to those found between anastomosing cells in embryonal hearts. Seiffert considered the spaces to be intracellular and also called attention to the spider-like cells. He compared these cells with the spaces to huge embryonic cells. Virchow [21] was not certain whether the spaces were lymphatic cavities or clear serous cavities. Hlava [22] believed them to be intracellular artifacts produced by alcohol during fixation. Ponfick, Knox and Schorer and Wolbach considered them to be intracellular.

The presence of glycogen in the vacuolated spaces has been a source of dispute as it is dissolved by solutions used in the ordinary methods of preparing sections. Seiffert was convinced of its presence but could not prove it. Rehder and Mönckeberg demonstrated glycogen in the tumor cells. Farber also demonstrated glycogen in the vacuolated spaces in the tumor in his case and states that others also have found this to be true. We were unable to demonstrate any in the vacuolated spaces of the tumor in our case.

Twenty-nine cases (57 per cent) of congenital rhabdomyoma of the heart were associated with tuberous sclerosis of the brain. Ponfick (1901) was the first to call attention to the associated cerebral sclerosis. He believed that the tumor of the heart and the sclerosis of the brain were congenital. Bonome felt that the association of rhabdomyoma of the heart with cerebral sclerosis was dependent upon the same conditions in intrauterine life, *i.e.* disturbances of nutrition with resulting vascular lesions. Steinbiss also noted the coexistence of rhabdomyoma of the heart with cerebral tuberous sclerosis. The latter may result in imbecility and epilepsy and accounts for the frequent occurrence of the tumor in inmates of hospitals for the insane. The cases reported by Steinbiss ranged in age from 5 to 35 years and all occurred in the insane.

Summary

1. The 51st case of congenital rhabdomyoma of the heart has been presented. The tumor occurred in a male infant who lived 3 hours after birth. Multiple tumors were found in the right

ventricle and interventricular septum. The tumor was characterized by many, usually rounded vacuolated spaces. The tissue between them was composed of large polygonal branching cells with a finely granular and eosinophilic cytoplasm. Congenital developmental defects of the heart as well as tuberous sclerosis of the brain with spongioblastoma were found in association with the heart tumor. As evidence of the congenital nature of rhabdomyoma of the heart, its occurrence in the newborn and its association with congenital anomalies have been noted. The true nature of the tumor is not known.

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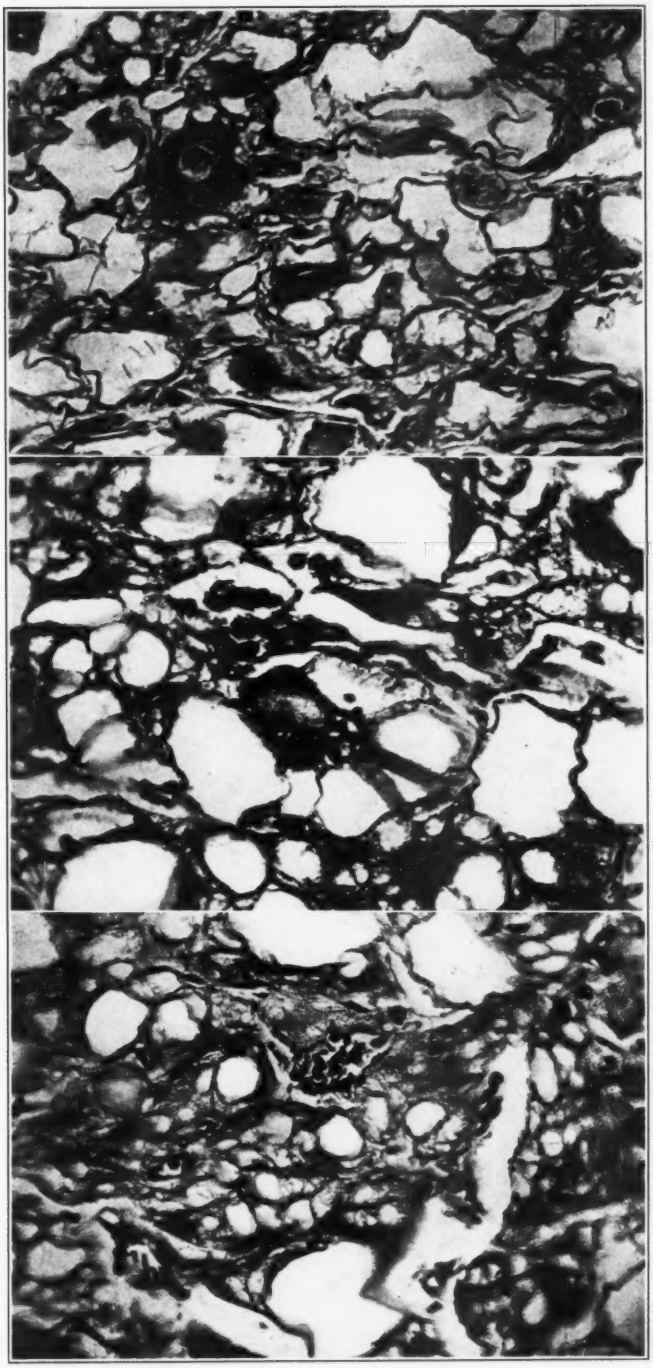
DESCRIPTION OF PLATE

PLATE 28

FIG. 1. Section from tumor showing the granules arranged to produce cross striations. Note the large number of vacuoles. Hematoxylin-eosin stain.

FIG. 2. Note the giant spider-like cell, the cytoplasm of which contains granular cross striations, in the vacuolated space. Foot's modification of the Masson trichrome stain.

FIG. 3. Large intracellular cells are seen occupying the vacuolated spaces. This is a characteristic feature of the tumor. Hematoxylin-eosin stain.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A METHOD FOR THE IMPREGNATION OF PERIVASCULAR NERVES ON INTRACEREBRAL BLOOD VESSELS *

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Study of the perivascular nerve supply of intracerebral blood vessels has always offered many obstacles. Although a wealth of silver impregnation methods is available, they all fall short of demonstrating adequately perivascular nerves on intracerebral blood vessels. In a quantitative study of the richness of the perivascular nerve supply to intracerebral blood vessels the Penfield [1] method was at first used. It was found, however, that although in many instances a clear picture was given, many specimens were lost from over-precipitation of the stain. In the search for a more consistent method the use of activated protargol was attempted. The method as originally described by Bodian [2] for other nerve structures was found to impregnate fibers on intracerebral blood vessels in tissue from animals freshly injected, but only an occasional fiber was impregnated on old, fixed human material. The following modification of the fixative used for fresh animal tissues, and treatment for old, formalin-fixed human material yielded excellent results.

The routine method is as follows:

A. Human Material Previously Fixed in 10 per cent Formalin:

1. Dissect out the vessels under the dissecting microscope.
2. Wash vessels in distilled water for 12 hours.
3. Place for 4 hours in a solution of alcohol, acetic acid and formalin made up as follows:

Alcohol, 95 per cent	90 cc.
Acetic acid (glacial)	5 cc.
Formalin	5 cc.

4. Wash for 2 hours in distilled water.
5. Place in 1–2 per cent aqueous solution of protargol containing 2 gm. of metallic copper (Merck) per 20 cc. of solution. Leave

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in this solution for 6–24 hours at 35° C., depending on the intensity of impregnation desired.

6. Wash in 3 changes of distilled water.

7. Place in the following solution until the vessels are a golden brown.

Hydroquinone	5 gm.
Sodium sulphite	10 gm.
Distilled water	100 cc.

8. Wash in 4 changes of distilled water.

9. Place in a 1 per cent solution of yellow gold chloride containing 3 drops of glacial acetic acid per 20 cc. until all color disappears from the vessels.

10. Wash in 2 changes of distilled water.

11. Place in a 2 per cent solution of oxalic acid in 1 per cent formalin until the specimens turn a light blue color.

12. Wash in 2 changes of distilled water.

13. Fix in 5 per cent sodium thiosulphate ("hypo") for 5 minutes and then wash in distilled water.

14. Treat with 95 per cent and absolute alcohol and clear in oil of Bergamot. The vessels are then flattened out under the dissecting microscope and mounted in balsam.

B. Fresh Tissue from Animals:

The brain of the animal should be washed through at first with physiological saline immediately after death until the fluid that comes from the jugular vein is perfectly clear. Then inject 500 cc. of the fixative given in Step 3. After approximately ½ hour the brain should be removed and placed in a fresh solution of the same fixative where it should be left for 24 hours. The vessels are then dissected free from the cortical substance, washed in 3 changes of distilled water, and impregnated as detailed from Step 5.

By the use of this modification it has been possible to impregnate consistently the perivascular nerve supply of intracerebral blood vessels in approximately 100 per cent of specimens examined, as reported in an article [3] to be published shortly. The richness of the vascular nerve supply has been shown to be much greater than before suspected. The fibers are shown uniformly as moniliform structures surrounded by a sheath containing the cells of Schwann.

By variations in the length of time the vessels are left in the protargol solution, and in the time allowed for development in the hydroquinone solution, it is possible to show clearly the smooth muscle cells in the media of the vessels. The minute structure of the muscle cell nuclei showing the depression in the nuclear membrane, termed the diplosome by Maximow,[4] and the condensation of nuclear chromatin material, as described by Boeke,[5] are clearly demonstrated.

Summary

A method whereby it is possible to demonstrate consistently the perivascular nerve supply of intracerebral blood vessels and the character of the blood vessel wall is presented. The illustrations show the clear picture given by use of this method.

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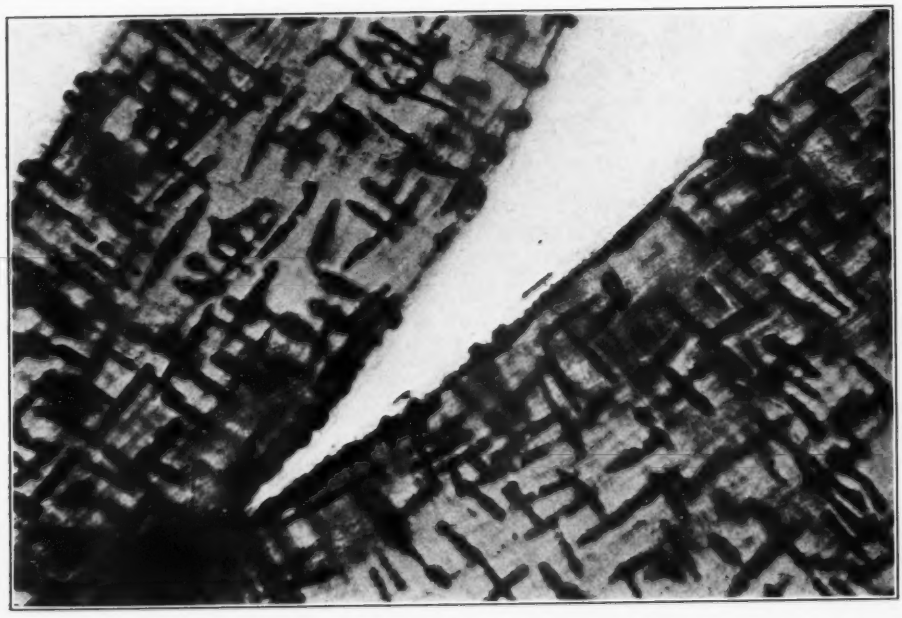
DESCRIPTION OF PLATE

PLATE 29

FIG. 1. Section showing a perivascular nerve fiber in an intracerebral blood vessel. The moniliform fiber and the sheath of Schwann with nuclei are clearly defined.

FIG. 2. Smooth muscle cell nuclei in the media of intracerebral blood vessels are clearly defined. Diplosomes and nucleoli are well differentiated.

1



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Humphreys Impregnation of Perivascular Nerves